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A Summary of Current Program 7/1/65

and Preliminary Report of Progress

for 7/1/64 to 6/30/65

ENTOMOLOGY RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

and related work of the

STATE AGRICULTURAL EXPERIMENT STATIONS

Section B

This progress report is primarily a tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of progress on USDA and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of USDA and cooperative research issued between July 1, 1964, and June 30, 1965. Current agricultural research findings are also published in the monthly USDA publication, Agricultural Research. This progress report was compiled in the Entomology Research Division, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Md.

UNITED STATES DEPARTMENT OF AGRICULTURE

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AREA NO. 13. BEEF CATTLE, HORSE, AND SWINE INSECTS

Problem. Insects and ticks irritate and torment livestock throughout the year in all parts of the United States and cause serious losses. These pests reduce weight gains, lower the quality of meat and hides, and spread numerous animal diseases. Livestock losses directly attributable to insects and ticks are estimated to exceed $300 million annually. Practical but not adequate control methods have been developed for lice, screw-worms, ticks, bots, grubs, and other insects, but satisfactory methods of protecting cattle from horse flies, deer flies, stable flies, mosquitoes, and the newly introduced face fly remain an unsolved problem. The development of insecticides for use on beef cattle, horses, and swine has been hampered because certain insects have become resistant to various insecticides, and because harmful residues have been found in meat following the application of certain materials. Safe, effective, nonresidue-forming insecticides and repellents are required. Urgently needed are economical and long-lasting insecticides or repellents for range cattle to protect them against vicious biting flies. Safer, cheaper, and more effective systemic insecticides and more efficient means of administration are needed to combat grubs and bots in cattle and horses. New approaches to control, including radiation and chemosterilants, should be explored to determine their feasibility as practical control methods. Efforts also need to be made to find and evaluate insect pathogens, parasites, and predators for controlling certain livestock pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycles that will serve as a basis for the development of more effective and safer methods of control. Research also is urgently needed on the role of insects in the spread of diseases of livestock.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing, long-term program involving basic and applied research on insects and ticks which affect the health and productivity of beef cattle, horses, and swine. Studies are conducted on the biology, physiology, genetics, and nutrition of the screw-worm, stable fly, horn fly, house fly, mosquitoes and other pests; on the nature of insect resistance to insecticides; and on absorption, metabolism and excretion of insecticides by insects feeding on or in animals; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is concerned with the development of more effective contact and systemic insecticides and protective treatments for the control of livestock pests. Studies are conducted to determine the occurrence of residues in tissues of animals treated with insecticides. Minor consideration is given to the development of sanitation and management procedures and biological control methods, including parasites and predators, for controlling the face fly, stable fly and several other pests. Emphasis is given to the development of insect sterility, attractants, and various other

noninsecticidal approaches to control. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to evaluate various kinds of traps and devices for estimating and controlling natural insect populations, and improved or special equipment for the application of insecticides to animals. Limited research is conducted on the role of insects and ticks as vectors of livestock diseases, with particular emphasis on bovine anaplasmosis and equine piroplasmosis. Research is conducted at McNeese State College, the University of Southwestern Louisiana, and the University of California under contracts and at the University of Wyoming under a grant.

The Federal scientific effort devoted to research in this area totals 17.8 professional man-years. Of this number,7.6 is devoted to basic biology, physiology and nutrition; 3.9 to insecticidal and sanitation control; 2.0 to insecticide residue determinations; 0.2 to biological control; 2.0 to insect sterility, attractants and other new approaches to control; 0.2 to the evaluation of equipment; 0.8 to insect vectors of diseases; and 1.1 to program leadership.

The Federal support devoted to research in this area under contracts and grants totals 1.2 man-years. Of this number, 0.5 is devoted to basic biology, physiology, and nutrution; 0.3 to insecticide and sanitation control, and 0.4 to biological control.

PROGRAM OF STATE EXPERIMENT STATIONS

Research on insects affecting livestock is an important part of the entomological program in the States. Studies on the distribution of pests and their relative importance under the varying conditions in different parts of the country form the basis for more exhaustive research on problem species. Biological information is being obtained in studies of habits, breeding sites, seasonal occurrence and dissemination. Such phenomena as host selection, feeding, behavior and host-parasite relationships are investigated by observing and collecting insects from hosts exposed in various habitats. The effects of livestock management practices on insect infestations are being determined by rotating pastures, varying forage types, and stocking rates and shelter manipulation.

Insecticides are being screened to determine their effectiveness as pest toxicants and safety. Systemic insecticides are receiving particular attention. Residue studies are being conducted to obtain basic information on the metabolism of chemicals in animals. Research is underway to determine the interrelationships in the metabolism of combinations of pesticides and to study the physiological and nutritional factors that influence the amount of tissue storage of insecticides.

Insect repellents, attractants and substances interfering with development are being identified and their effectiveness in control evaluated. Information obtained is being considered to determine the feasibility of using

such techniques in combination with chemosterilants.

A total of 21.9 professional man-years are devoted to this area by the States.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies were continued on the biology of Anopheles quadrimaculatus by artificially augmenting the population in an isolated breeding area. It was shown that: (1) the density of the population was increased in the study area when a constant breeding area was supplied to egg-laying females and when a blood source was provided, (2) wild females preferred natural ponds as an egg-laying site but used artificial ponds when natural areas were not present, and (3) Anopheles quadrimaculatus adults preferred resting boxes which were painted black and placed on a horizontal plane.

New rearing diets and rearing techniques were evaluated for Anopheles quadrimaculatus. Rye grass infusion or extract in the rearing medium accelerated larval development. There was a positive correlation between the number of larvae per rearing pan and larval mortality. Protozoans were an important source of food for the larvae. A rearing method incorporating all of these factors increased survival and size of the insects and decreased the time required for development and their tolerance to insecticides. Similar results were obtained with four other species of mosquitoes, Aedes aegypti, A. taeniorhynchus, Culex quinquefasciatus, and Aedes triseriatus.

A new method of separating pupae of Anopheles quadrimaculatus from larvae has been adopted. When mixtures of both are placed in ice-water, larvae sink and pupae float allowing rapid separation through the use of a funnel. Time required to separate the stages in colony production was reduced 86%.

At Corvallis, Oreg., studies were continued on the biology of the mosquito, Aedes increpitus, in the Willamette Valley. During the winter months of the last three years, larvae of this species have been collected in numerous habitats of the flood plain of the Willamette River. Following an unusually protracted period of subfreezing temperatures during which a low of 8° F was registered and near-record floods during which all low lying areas in the Willamette Valley were inundated for several days, larvae could be readily collected. The strain of increpitus in the Willamette Valley apparently has become well adapted physiologically to the rigors of the area over a long-term period of time. In other studies, a strain of Culex pipiens quinquefasciatus, which is orange in color as 4th-instar larvae and newly formed pupae, has been isolated. The strain has bred true for 3 generations and appears to be genetically recessive.

At Lake Charles, La., basic studies on the biology of floodwater mosquitoes was conducted. Studies have shown the comparative longevity, blood-feeding, and oviposition patterns of different species of flood-water Aedes species. Aedes taeniorhynchus, A. sollicitans, and A. infirmatus are more important as pest mosquitoes than other species that occur in the area. Studies on the amount of blood ingested by 12 pest mosquito species occurring in Southwestern Louisiana indicated that females of all species ingested sufficient blood at one feeding to at least double their body weight. Psorophora cyanescens, Aedes atlanticus-tormentor, and Anopheles quadrimaculatus, more than tripled their body weight with blood from one feeding.

Research has been conducted under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the production, relative abundance and dispersal of pest mosquitoes in the Gulf Coast area of Louisiana. Data has been maintained on rainfall and temperature in relation to mosquito production and some information has been obtained on the influence of rains versus tidal action in mosquito production. An impoundment is being developed to study the effects of impounding and water management procedures on mosquito production in the area.

2. House Fly. At Gainesville, Fla., research was continued on basic biology of the house fly. Evidence was obtained that an olfactory attractant, or pheromone, specific for the males of Musca domestica L. is not produced only by the females. The attractant was found on contaminated holding cylinders and on dead and non-virgin females. In addition, live males were also somewhat attractive. The degree of attraction was of a low order, resembling other reported sex pheromones of the house fly. This attractant was soluble in methane and slightly soluble in benzene. Data also showed that the time of day at which pupal eclosion occurs is influenced by photoperiod, but photoperiod may not be the only controlling factor. Response to insecticides was also shown to be regulated by photoperiod.

In mating experiments, female house flies mated more readily with males from their own strain than with those of other strains. When normal females from the latorabory or Grand Turk (wild) strains were confined with normal males from one strain and chemosterilized males from the other, they mated more readily with males of their own strain, whether sterilized or not. Chemosterilized males competed more successfully than normal males of the same strain.

At Corvallis, Oreg., research was conducted on the genetics and physiology of house flies and data developed in these studies were used to elucidate mechanisms of insect resistance to insecticides.

Several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. Several of the mutant strains have been defined genetically and are being maintained. Most mutants involved wing form, wing positioning, or pattern of wing venation. Three established mutant strains, classic wing, stubby wing, and dot vein have

proved useful in the genetic analysis of insecticide resistance. For example, two DDT resistant strains of house flies were found to possess a fifth chromosomal dominant which confers moderate resistance to DDT, but does not confer resistance to o-chloro DDT. In addition, one of the strains possessed a second chromosomal recessive which conferred moderate resistance to DDT and high tolerance to o-chloro DDT. The presence of both factors in a strain confers virtual immunity to DDT. Substrains were isolated, each possessing only one major factor for resistance and the nature of the two independent factors for resistance confirmed through appropriate crosses and bioassays. Resistance associated with the fifth chromosomal dominant is that for DDT dehydrochlorinase. The mechanism of resistance associated with the second chromosomal recessive is unknown, but apparently does not involve dehydrochlorination.

Physiological studies of mechanisms of resistance to organophosphorus insecticides in house flies showed that blocking of ali-esterase with a selective inhibitor increased the accumulation of paraoxon and also the toxicity of parathion and paraoxon in both susceptible and parathion-resistant strains. These results indicated that ali-esterase is an important detoxifying enzyme. The role of ali-esterase in organophosphate poisoning appeared to be related more to detoxication of paraoxon than of parathion in both susceptible and parathion-resistant house flies.

A resistance factor (esterase(s)) for parathion in house flies was shown to be transmitted in a dominant manner and was at least 1000 times less sensitive to inhibition by paraoxon than a corresponding esterase present in a susceptible marker strain.

In other studies successful mating of house flies kept in complete darkness from the time of emergence from pupae to separation of the sexes occurred. Sex ratios were normal among the offspring.

3. Stable Fly. At Gainesville, Fla., studies were continued on the stable or dog fly, a serious pest of animals and humans along the northwest coast of Florida. A general outbreak of stable flies occurred during the last week of August and the first 2 weeks of September in 1964. Population counts around motels and other structures and on beaches ranged from landing rate counts of 2 to 94 flies per man per minute. Bay grass deposits in 1964 were much heavier in 1964 than in 1963. Observations indicated that the insects developed in the bay grass in about the same length of time as was required for development in CSMA medium under laboratory conditions.

Tests indicated that the effectiveness of insecticides against stable flies varied with the time of exposure after blood feeding. Other research in large outdoor cages indicated that landing rate counts of stable flies were a good index of total populations since the same percentage of flies landed on human observers when the total number of flies was varied.

In Nebraska the exposure of successive generations of stable fly larvae to DDT in the larval medium resulted in a gradual increase in tolerance to the insecticide. By the 39th generation tolerance had increased by 45-fold, but by the 42nd generation tolerance had declined to about 2-fold. The reasons for this rapid decline have not been determined.

In Texas studies were conducted to determine the effects of 4 different conditions of light (continuous light or dark, 12 hours light and 12 dark, and normal daily fluctuation) on the pattern of emergence of adult stable flies from pupae. Emergence in all tests was essentially circadian in pattern, with peaks between 6 and 9 a.m. The bulk of emergence in each group occurred on the 3rd, 4th, and 5th days after first emergence.

In Texas studies were conducted to determine the function of the antennae in feeding and mating behavior of male and female stable flies. When the antennae were completely covered with Plexiglass glue the flies did not feed and mating was greatly reduced. Additional tests showed that blinding the flies by covering the ocelli with black paint also greatly reduced mating. These results indicate that the antennae play an important role in feeding and mating responses of flies and that sight (i.e., light) is necessary for maximum mating response. Further studies showed that when stable fly pupae were held in total darkness, adults emerged and fed but only a small percentage mated and produced viable eggs.

4. Face Fly. In Nebraska, laboratory studies on the reproduction of the face fly showed that as the proportion of males to females was increased, reproduction per female decreased. This was true when only fertile males were mated with the females as well as when various ratios of fertile and sterile males were used. Decreased reproduction apparently was due to harassment by males which reduced female longevity and thereby reduced oviposition.

Studies were conducted under laboratory conditions to determine the egg-laying pattern, number and fertility of eggs and longevity of laboratory reared face flies. Female flies were allowed to mate once then confined individually in small cages for observation. Longevity of the flies ranged from 9 to 60 days, averaging 28.4 days. Batches of eggs per female ranged from 0 to 9 and the number of eggs per female varied from 0 to 181, averaging 56.6. Viability varied from 0 to 89.0%, averaging 60.2%. A few flies oviposited when 4 days old but the average age was 10 days. Results of these tests indicate that multiple matings may be necessary to insure sufficient spermatozoa to insure fertilization of all eggs laid.

Preliminary studies with 3 different strains of face flies showed very little difference in the longevity, fecundity, and rate of development of the colonies maintained under continuous light and under 16 hours of light and 8 hours of darkness. No circadian rhythms were observed but studies are being continued.

In Nebraska studies were conducted to determine the pattern and distance of dispersal of marked, laboratory reared flies released in the field. Observations indicated that the flies dispersed rapidly in all directions. Maximum distance of recovery from the point of release was 1.4 miles after 24 hours. After 10 days a few marked flies were observed at the release site.

Face flies were first observed in the field in May but populations remained low until late July, whereas in 1963 high populations developed by mid-June. Populations on cattle remained fairly constant in August but fluctuated with changes in temperature in September. When temperatures were below 70° F very few flies were active. Maximum populations of 13 per cow were noted on September 29, a warm day, but on this date flies began to hibernate in one location (grain storage building) although the inside temperature was 90° F. However, the flies moved in and out of the building and small populations persisted on cattle until the advent of cool weather in late October.

Studies were continued in 1964 on the insect fauna in cattle droppings on 3 of 8 farms studied in 1963. Over half of the insects collected in droppings were Diptera, 42% of which were face flies. The total insect population in 1964 was substantially higher than in 1963 but the number of face flies in droppings was almost identical. Parasitism of face fly pupae was low (0.7%) in 1964 but adult populations on cattle were constantly lower than in 1963. Reasons for this were not determined. In addition to face flies, droppings contained substantial numbers of aphodius beetles and Sarcophaga larvae. About 16% of Sarcophaga pupae were parasitized by Hymenoptera and Staphylinidae. Collections showed 10 species of Diptera, about 30 species of Coleoptera, and 3 species of parasitic Hymenoptera.

In Maryland, outdoor behavior of face flies was studied, both on herds in the field and on a single animal confined in a cage with a known fly population. The data indicates the following: Only a small proportion, usually less than 10-15% of the total face fly population actually annoys cattle at any given time. Female flies visit the animals much more frequently than males, but males do cause some annoyance. Three- and five-day-old females visit the cow more frequently than 1-day-old females. The number of flies present on cattle is most closely related to the activity of the animal, with the greatest annoyance when the cattle are resting quietly. Although flies visit the face most frequently, they do rest on other parts of the body in considerable numbers. An evident peak of activity of released colonized flies occurred early in the morning, but that of wild flies appeared more evenly throughout the day. All flies leave the cattle at dusk while natural light levels are still quite high.

Additional studies of the nocturnal habits of face flies confirmed that they rest on the foliage of trees at night. Although the flies are readily attracted (about 80%) to blacklight in confined spaces, those found resting on foliage at night do not respond in this manner. Electrocutor grid traps with blacklight lamps placed in trees attracted less than 1% of a released

population in 48 hours. Investigations of the factors affecting this change in behavior may provide information useful in control.

Laboratory tests of the mating activity of female face flies indicate that sterilized males compete effectively with normal males in mating. Also, females appear to mate only once if they are inseminated during their first mating. Examination of females observed attempting to remate showed that only 5-10% of all females had not received any sperm during their first mating. This characteristic indicates that use of sterilized males should be effective in preventing reproduction. A laboratory trial using a ratio of 8 sterilized males to 1 untreated male to 1 untreated female resulted in a 94% reduction in pupae.

When marked face flies were released near cows in Maryland about 1 hour before sunset, a few marked flies and a few wild flies were noted on the cows for several minutes after release. They left the cattle when the level of natural light was relatively high, indicating that artificial light would probably not be an effective attractant. In dispersal studies, 24,000 individually marked face flies were released in 4 different areas. Several marked flies were found 2 miles from their release point after 24 hours. One fly was found 4 miles away after 5 days. Searches for marked flies during the late fall showed that they spend the night in trees and tall weeds rather than in or around barns.

5. Horn Fly. In Texas a number of adult horn fly diets were compared for suitability in maintaining laboratory colonies. Diets composed of bovine blood, a saline extract of ground beef muscle and antibiotics or bovine blood, ACD, and cholesterol proved most satisfactory. Flies consumed more, reproduced better, and survived longer on these than any other diets tested, including the standard (citrated blood, tissue fluid, and antiobiotics). Liquid extracts from manure of cattle fed fresh oat and dry alfalfa, sorghum, and prairie hay were poured over cotton gauze pads and compared for suitability as horn fly larval media. The oat and alfalfa extracts produced pupae that averaged 3.16 mg in weight as compared to 2.5 mg and 2.1 mg for those reared on sorghum and prairie hay extracts, respectively. In additional tests in which the pH of the media was adjusted, horn fly larvae survived and developed equally well when pH's ranged from 6.0 to 9.0 but none developed in pH's 5.5 or less or 9.5 and above.

In Texas studies showed considerable variations in the color of eggs deposited in manure by horn flies. Counts indicated about 84% of the eggs were dark brown, 13% tan, and 3% yellow and white. The eggs were equally viable and equally capable of producing progeny.

In Texas pressuring of successive generations resulted in a gradual increase in adult tolerance to ronnel. By the 20th generation a dosage of 0.1 ug/fly caused only 32-56% mortality. By the 28th generation tests showed an LD 50 of 0.3 ug/fly or about 50 times that of a normal susceptible strain.

6. Screw-worm. Research was continued on the screw-worm fly at Mission, Texas, in support of Southwestern screw-worm eradication program. Special studies were continued to develop a strain of flies resistant to starvation. Continuous selections resulted in a gradual increase in resistance to starvations until only negligible mortalities occurred in 72 hours in the 19th generation, in 96 hours in the 36th generation and in 144 hours in the 40th generation. Substrains removed from selection in the 18th and 30th generation retained their ability to withstand starvation. The sexual vigor of starvation resistant flies decreased gradually as resistance increased but the substrain removed from selection showed almost normal vigor 7 and 9 generations later. When females of the 27th generation of the resistant strain were held with males of the same strain under 96 hours of starvation no viable eggs were produced. When females were fed, the hatch of eggs was 38% as compared to only 14% when only males were fed. When both sexes were fed hatch was 57% or about the same as that for fed unselected females and males.

Studies were continued on the mating behavior. It has been generally believed that female screw-worm flies mate only once; however, close observations showed that a high percentage of the female flies that mated with 1-day old males (immature) mated a second time with mature males. On the other hand only 2 of 110 females first mated to mature males remated, 1 willingly and the other under duress. Egg viability was only 24% when females were mated with 1-day old males but increased to 65% when females were remated with mature males. When 1-day old males were exposed for 8 hours with 3-day old (mature) females and then replaced with mature males, egg hatch was only 52%, compared to 89% for matings of mature males and females. These results indicate that females seldom remate if their first mating is satisfactory, i.e., with a mature male.

Competitiveness of irradiated (R) and non-irradiated (N) males was compared in multiple mating of females. Frequency of remating was increased by subjecting females to starvation periods of 20 to 24 hours and all matings were confirmed by observation. Females mated first with R males and then with N males averaged 33% to 48% fertility in 2 tests, compared with 75% to 85% for the controls. However, in the reciprocal matings fertility was 80% in both tests, indicating that R males were not competitive with N males. Mortality and fecundity of starved controls subjected to single matings were normal; however, 33-71% of the remated females failed to survive. Actual cause of death following forced second matings has not been determined but these observations help to clarify SAG test results in which aggressive males greatly accelerate female mortality.

Occasionally mating and fecundity studies have been conducted in which oviposition was induced immediately after copulation. Since duration of sperm storage in some mammalian females greatly affects fertility, this possibility was investigated in screw-worms. In 2 out of 3 tests fertility ranged from 47% to 55% when oviposition occurred within 3 hours of copulation, compared with 78-86% at 24 hours. In a third test fertility was 59% within 4 minutes but ranged from 80% to 97% from 3 hours to 4 days. Although

the influence of sperm storage on fertility has not been clearly defined, a minimum 24-hour period between copulation and oviposition has been adopted in mating and fecundity studies.

Three screw-worm males selected at random from the Florida colony mated a total of 72 females each. All matings were confirmed by observation during daily 4-hour sessions. Two males ceased mating at 7 days when excessive wing damage appeared to interfere with proper positioning, and the other was dead on the 7th day. Peak mating activity occurred during the 3rd to 5th days. Total matings ranged from 1 on the 1st day to a maximum of 20 on the 3rd day; however, from the 3rd to 6th days fertility primarily occurred only among the first 7 matings. Total females fertilized per male (hatch 1% to 98%) ranged from 18 to 24. These results are in agreement with previous studies in which mating activity was evaluated only on the basis of hatching records. Although female remating seldom occurs following copulation with a mature, spermatous male (unless the females are too weak to elude the male), almost 70% of the females in the above test remated.

Studies were conducted to compare the ovarian growth of normal and starvation-resistant female flies under starvation, feeding after starvation and continuous feeding. Under starvation very little growth occurred but when food was provided the starvation-resistant females showed faster development than those of the normal strain. Ovarian growth was comparable when the two strains were fed continuously. Ovarian growth was more rapid in females fed meat than in those on a meatless diet.

Bioclimatic studies showed that both normal and selected strains of newly emerged screw-worm flies survived well when held 30 to 45 hours at 32° F, mortalities being 25%and 10%,respectively. Oviposition and egg viability of surviving females was not affected by the exposure. Exposures of 48 to 72 hours at 105° F caused 46 and 38% mortalities of the two strains and oviposition by the survivors and viability of eggs was greatly reduced.

Laboratory studies showed that male screw-worm flies reared on horse meat were about 25% larger (based on weight) than flies reared on the liquid medium now used for mass production of screw-worms for use in the Southwestern control program. Also the meat-reared males were able to mate with 40% more females than the liquid-medium reared flies, although no difference was observed in the number of mating attempts by the two strains.

A preliminary investigation made in January in Mexico indicated that in average winters there is very little screw-worm overwintering in the northwestern corner of Sonora or in the northern part of Lower California. However, in the coastal regions of Sonora between parallels 28° and 30°30', it is seldom cold enough to completely eliminate screw-worms. Instead, they are confined to certain bowl-shaped terrain features known locally as bahias. These bahias are warm and moist and furnish preferred pasture for cattle both winter and summer. It appears that the bahias have somewhat the same

relation to screw-worm survival as the river valleys in southwest Texas, but it is not known to what extent screw-worms move from one bahia to another at different times of the year.

In Texas field tests were conducted to study the relative dispersal abilities of irradiated normal and selected (starvation-resistant) strains of screw-worm flies. A total of 330,000 flies of each strain were distributed by airplane in two releases along a 6-mile swath on a large improved range area. Totals of 1659 selected strain flies and 2252 normal flies were recovered from traps, indicating that the normal flies were more vigorous than those of the selected strain.

In August 1964 a test was initiated in a 2000-square mile area in Veracruz, Mexico, to determine the efficiency of dispersal of flies dropped from aircraft at 8-mile swath intervals as compared to the standard intervals of 2-4 miles. Eleven releases of 400 males per square mile were made between August 29 and November 6. Since the area was naturally infested with screw-worms, efficiency was based on the percentage of sterile egg masses collected on wounded animals in pens located 0.1, 2 and 4 miles from release lines as compared to that on animals in a control area. Fly traps were operated at each pen from October 19 until December 1 to obtain data on the relative abundance of wild and released flies. The number of egg masses was fairly high early in August but declined rapidly with the onset of hot-dry weather and remained fairly low until late September. The numbers of egg masses began increasing with favorable weather early in October and remained fairly high until termination of the test. A few sterile egg masses were collected after the first male release. The percent sterility increased steadly thereafter to a peak of 68% by October 2 and declined gradually to about 20% at time of the last fly drop on October 27. There were no significant differences in egg mass sterility at different distances from lines of dispersal, indicating that the flies dispersed uniformly and apparently rapidly. All trap catches showed larger numbers of sterile flies than wild flies from October 26 through November 6 but native flies outnumbered sterile flies in all traps by November 14. Only 2 sterile flies were present in collections on November 19 indicating a maximum longevity of about 3 weeks.

7. Cattle Grubs. In Texas studies were continued to develop laboratory techniques for the rearing of cattle grub larvae. Several media consisting of agamma calf serum combined with various chlorides, glucose, and other materials proved highly satisfactory for in vitro rearing of cattle grub larvae. Some first instar larvae survived as long as 120 days in these media and many doubled or tripled their weight during this period. Small numbers molted to the second instar and survived another 60 days.

A major problem in in vitro rearing of cattle grub larvae is the development of bacterial infections which kill the larvae and considerable effort was devoted to the development of ways and means to solve this problem. Ultraviolet light effectively controlled bacteria but caused high mortalities

of grub larvae. Various antibiotics and merthiolate were partially effective but reduced longevity of larvae. Studies are continuing.

Studies were undertaken to determine the absorption and ingestion of food by 1st instar cattle grub larvae confined in artificial media containing P^{32}-labeled phosphate. Examinations showed that the larvae contained much higher concentrations of radioactivity in the blood and integument than in the gut at any given time interval, indicating that most if not all intake was by absorption. Similar results were obtained with media containing dyes or carbon black. Dissections of larvae and examinations of the foreguts and hindguts failed to reveal any evidence of a lumen.

In Texas all grubs from the backs of a number of cattle imported from Wyoming were extracted and identified. The population consisted of 83% H. lineatum and 17% H. bovis.

8. Horse Flies and Deer Flies. In Mississippi, studies were conducted to develop a suitable medium for the rearing of tabanid larvae in the laboratory. Sand and soil was unsatisfactory because larvae did not survive and develop well and they could not be observed without removing them. A semi-solid agar medium proved fairly satisfactory but it tended to harden with age. Of other media tried the most satisfactory consisted of small glass beads covered with water. The larvae survived well, were able to move freely, the container and larvae were easily cleaned, and the larvae were easily observed while moving and feeding on fly larvae or worms. From 60 to 85% of larvae of T. americanus, T. abdominalis, T. proximus, and C. crepuscularis survived by this rearing technique. Survival of 4 other species was much lower. Larvae of T. vittiger schwardti showed the most rapid development from egg to mature larvae. Length of larval instars ranged from 5-6 days for the 1st, 2nd, and 3rd to 52 days for the 7th. The average time from egg to mature larvae was approximately 120 days. Attempts to mate adults were unsuccessful.

In additional tests larvae of T. vittiger schwardti developed from the 1st through the 4th stage fairly rapidly in from 5.0 to 11.3 days per stage but development of succeeding stages required successively longer times, ranging from 18.3 days for the 5th to 60.5 days for the 8th stage. Length of the pupal stage ranged from 5 to 10 days, averaging 7 days. Total days from egg to adult ranged from 143 to 209 days, averaging 189 days.

In Mississippi, after many unsuccessful attempts, a method was developed for obtaining eggs from engorged adult tabanids. Engorged adults are placed in a screen cylinder which is then inserted into a wide-mouth gallon jar containing 2-3 inches of water-saturated sand covered with a screen shield. The upper end of the screen cylinder projected 4-5 inches above the mouth of the jar and is covered with cheesecloth. This arrangement provides the range in humidity necessary to satisfy the different water requirements of various species. With this arrangement eggs were obtained from 8 species and records made of the numbers of egg masses laid and days to hatch. The

number of egg masses ranged from 1 for T. atratus and 4 other species to 10 for T. abdominalis. Eggs of T. lineola and T. vittiger schwardti hatched in 2-3 days but those of other species required from 5 to 7 days. Newly hatched larvae of most species survived and developed well in thin layers of agar in plastic dishes.

In Mississippi, adult horse fly populations increased gradually during the spring, reached a peak during late May and early June, and declined rapidly during late June and early July. Seven species were present during this period but the predominant species was T. vittiger schwardti followed by T. lineola, T. fuscicostatus, T. equalis, T. abdominalis, T. americanus and C. crepuscularis.

B. Insecticidal and Sanitation Control

1. Mosquitoes. In Oregon 56 compounds were screened on cattle by the "spot test" method for effectiveness as toxicants and repellents against adult mosquitoes. None of the materials were outstanding toxicants. Four materials--ENT 27194, ENT 27195, ENT 27196 and ENT 28086--showed fair to good repellency at dosages of 500-1000 mg/ft^2.

At Gainesville, Fla., the developmental program on insecticidal compounds for mosquitoes was continued. A large number of new candidate materials were tested in the laboratory for their potential as larvicides and adulticides. Many proved promising for further development.

Tests were conducted to evaluate fogs of naled, fenthion, Bayer 39007, and malathion against caged salt-marsh mosquito adults. Bayer 39007 was the most effective in these tests followed by fenthion, naled, and malathion. A field test in which different formulations of malathion were applied by airplane at a ratio of 0.05 lb/acre showed a reduction of 81% in population levels with the fog oil formulation, 76% with fuel-oil formulation and 44% with water emulsions. Airplane spray tests with four organophosphorus insecticides on adult salt-marsh mosquitoes indicated all were highly effective at low dosage rates. Comparison of the effectiveness of aerial sprays of malathion applied as a thermal fog and fuel oil spray showed the latter to be more effective.

Tests were conducted in the rice-growing area near Stuttgart, Ark., to evaluate the residual effectiveness of some new insecticides against natural infestations of Anopheles quadrimaculatus. The insecticides were applied to the walls and ceilings of farm buildings at 200 mg/ft^2 as wettable powders and/or emulsions. Pre- and post-treatment counts were made of the mosquitoes resting in the treated buildings as well as in six untreated buildings which were utilized as checks. An emulsion of Hercules 9485 was highly effective, causing 99.7% to 100% reductions for at least seven weeks. As this compound was not available at the beginning of the series, it was applied from two to three weeks after the other treatments. A wettable

powder formulation of Shell SD-8530 caused reductions of 98-100% for 9 to 10 weeks. A malathion wettable powder used as a standard caused 100% reduction for 4 weeks and 96-100% reductions (average 99.0%) throughout the 9-week test period. Hercules 9326 emulsion caused 100% reduction of the mosquito infestations for at least 9 to 10 weeks in two buildings but in a storage shed produced only 91% to 96% control the 6th and 8th weeks. Wettable powder formulations of CELA S-1942 and CELA S-2225 were slightly less effective, with control falling below 70% in some buildings by the sixth week. Shell SD-8211 was highly effective in two buildings but not in a third.

Tests were also conducted to evaluate the residual effectiveness of treated cheese-cloth when applied to the walls and ceiling of buildings in the same area. The cheese-cloth, which was purchased in rolls 3 feet wide, was first flameproofed and then impregnated with Bayer 39007. Buildings in which a complete coverage of treated cheesecloth had been used showed 100% reduction of the mosquito populations for the full 10-week duration of the test. All buildings treated by means of a strip of cloth around the edge of the ceiling and in the corners showed 100% reduction for 5 weeks, and 82 to 99% control for the next 5 weeks. Buildings treated by means of cloth around the edge of the ceiling only, or in the corners only, showed 98 to 100% reduction of mosquitoes for 5 weeks, and 84 to 100% control for the next 5 weeks.

At Corvallis, Oreg., tests were continued on the development of more effective insecticides for mosquito control. In field tests against snow-water Aedes mosquito larvae, excellent results were obtained with lindane, BHC, and fenthion at 0.05-0.1 lb/acre. Abate and Dursban were generally less effective. Against mosquito breeding in log ponds, granular formulations of fenthion and abate gave excellent control. Both were also effective when applied with a pump oil can. In cooperative tests in California low volume airplane sprays of malathion and fenthion showed considerable promise as mosquito larvicides.

Infusions and hot water extracts made from several tree species were tested for toxicity against Culex tarsalis larvae. Toxic elements were found in Western red cedar, ponderosa pine, and to a less extent in lodgepole pine and redwood. Similar hot water extracts made from Douglas fir, Sitka spruce, Western hemlock, big leaf maple, red alder, and white fir were nontoxic to larvae. Studies are in progress to characterize the toxic principles through fractionation of extracts.

None of 7 analogs of DDT showed promise against resistant Culex tarsalis larvae.

At Corvallis, Oreg., experiments with C^{14}-TDE indicated that both susceptible and DDT-resistant Culex tarsalis larvae detoxified TDE by dehydrochlorinative and oxidative routes. The results suggested that resistance to DDT and related compounds in tarsalis involves a mechanism other than dehydrochlorination.

Studies were continued in the search for compounds that would act as synergists to overcome insecticide resistance in mosquitoes. Of a number of phosphorus esters, butyl-containing esters were most effective although other types showed activity.

2. House Fly. At Gainesville, Fla., research was conducted on the development of safer, more effective insecticides. Materials were evaluated in the laboratory as contact sprays and residual toxicants as a basis for selecting promising insecticides for field evaluations. Evaluations as residual toxicants included different formulations of the materials. Twenty-one promising compounds were tested as house fly larvicides in manure under caged poultry. Four compounds were highly effective as larvicides.

Residual tests were conducted with emulsions of malathion, diazinon, ronnel, dimethoate, naled, fenthion, and Bayer 41831, and with wettable powders of malathion and Mobil MC-A-600 against house flies in barns. All were applied at 100 mg/ft^2. Control was considered satisfactory as long as the reduction produced by the chemical was 75% or above. Dimethoate residues gave satisfactory control on most occasions for 14 days, after which they were ineffective. Mobil MC-A-600 wettable powder gave satisfactory control for 14 days in one test, but failed as early as the 1st day in a replication of the test. Other compounds gave satisfactory control for shorter periods.

At Corvallis, Oreg., research was continued to find compounds effective in synergizing organophosphorus insecticides and resistant strains of house flies. A number of different types of phosphorus esters were effective when combined with either malathion or parathion in overcoming resistance in house flies to those two compounds. Materials synergizing malathion against resistant insects differed considerably from those known to potentiate the toxicity of malathion to mice or cause ataxia in poultry.

3. Stable Fly. In Texas 119 new compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Nine of these compounds were Class IV toxicants at concentrations of 0.5% or lower. Materials effective at a low concentration of 0.1% were Shell SD-8967 and SD-9102, and CELA S-1942. The only effective repellents at a 5% concentration were ENT nos. 28086, 28087, and 28093. Special tung oil formulations from a commercial source proved both non-repellent and non-toxic to stable flies. Spot tests were conducted to evaluate a number of materials as extenders for pyrethrum and conventional insecticide. One material, Armour ARD-226, increased the repellency of malathion and extended residual effectiveness slightly. Other materials were ineffective.

In Texas, large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low volume sprays for the control of stable flies. Treated cattle were exposed for 24 hours periodically to flies in large cages but otherwise were kept outdoors. Pour-ons (8 oz/animal) of coumaphos were effective against stable flies for 15 days. Pour-ons of fenthion and ronnel were only slightly less effective, but carbaryl

was effective for only 3 days. Conventional 2-quart spray applications of 0.1% Stauffer R-5723 and 1% Mobil MC-A-600 and CELA S-1942 were effective 3 to 10 days against stable flies. In similar cage tests with low volume sprays (23-69 ml/cow), 1% applications of 0.5% methoxychlor, malathion and DDT controlled stable flies for 3-6 days as compared to 1-3 days for seven other materials.

Comparative tests were run with the WHO test kit to compare the susceptibility of stable flies to 12 insecticides. Five of the materials were equal in toxicity to ronnel (standard) and 3 were more toxic, namely, Shell SD-8436, SD-8447, and SD-8448.

At Gainesville, Fla., chemicals were evaluated in the laboratory as potential larvicides for the control of stable flies. Approximately 150 compounds were tested by exposing larvae to these compounds when they were incorporated into the larval rearing medium. Approximately 20 of the compounds were highly effective, approximately equal in activity to a standard, Bayer 39007. Tests with calcium arsenate as a larvicide indicated that it did not compare favorably with other compounds that were evaluated. In addition, approximately 120 compounds were evaluated in laboratory tests as adulticides and some 20 were highly effective. Several of the more promising adulticides were tested as fogs against caged adults under field conditions, indicating the potential of these materials for controlling natural populations. Comparative tests of adulticides against caged insects indicated no differences in effectiveness of thermal vs. nonthermal fog applicators or between fuel-oil and water-based formulations. A contract was negotiated with the Florida State Board of Health to conduct research on insecticides for controlling natural populations of stable flies under conditions found in the Gulf Coast area of Northwestern Florida. The research contract will take advantage of research conducted at the Gainesville laboratory and evaluate insecticides under practical field conditions.

4. Face Fly. In Nebraska bioassay tests showed that the addition of 1.0 ppm of Thiabendazole, a new parasiticide for livestock, to manure prevented the development of face fly larvae to the adult stage. Concentrations of 0.1 ppm and lower were ineffective. The addition of 0.5% of Bacillus popilliae to manure had no effect on the development of face fly larvae but the addition of 1.0% reduced fly emergence by 24%.

5. Horn Fly. In Texas large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low volume sprays for the control of horn flies. Treated cattle were exposed periodically for 24 hours to flies in large cages but otherwise were kept outdoors. Pour-ons (8 oz/animal) of coumaphos were effective against horn flies for 20 days. Pour-ons of fenthion and ronnel were slightly less effective and carbaryl was effective for only 3 days. Conventional 2-quart spray applications of 0.1% Stauffer R-5723 and 1% Mobil MC-A-600 and CELA S-1942 were effective for 7 to 10 days. In comparative tests low volume sprays of 5 and 10%

ronnel gave 100% kill of horn flies in 24 hours whereas 1% sprays gave only 86% kill.

In Oregon large cage tests were conducted to determine the effectiveness of certain tacky polybutanes against horn flies. All of the materials were repellent the first day after application but only one--Amoco H-120--showed repellency for 2 to 3 days. Additional cage tests were run to evaluate the effectiveness of 1/2 inch, 1 inch and 1 1/2 inch plastic collars containing 20% dichlorvos for the control of horn flies. The collars reduced horn fly populations by 86 to 100% in 3 hours and 100% in 24 hours and were still completely effective after 2 weeks of wear and exposure.

In the mid-coastal areas of Texas pour-on applications of 2 ounces per cow of 8% Ruelene and 5% ronnel gave excellent control of horn flies for 6 days, while applications of 4 ounces were effective for 11 days. In central Texas conventional sprays of 0.3% Ciodrin, 1% trichlorfon, 1% CELA S-1942, 0.5% carbaryl, and a pour-on of 1.0% coumaphos provided effective control for 2 weeks. Several other materials were effective for about 1 week. Similar treatments gave slightly shorter periods of control in humid coastal areas.

Low volume sprays of 5 and 10% ronnel applied to 1/ft^2 areas (withers and brisket) gave good control of horn flies but lower concentrations were unsatisfactory. In other tests excellent control of horn flies was obtained by treating only part of the animals in herds with 8% Ruelene at 1 oz/100 lbs body weight as a pour-on treatment. In one test the treatment of only 2 cattle in a herd of 50 reduced the overall horn fly population by 75% in 10 days.

In Texas bioassays were run to determine the toxicity to horn fly larvae of the manure from cattle that had been fed seven insecticides at varying rates for 10 days. All of the materials reduced larval survival but only Bayer 37341 and Stauffer R-3828 at 5 mg/kg daily gave 100% mortality.

In Mississippi, conventional spray applications of 2 quarts per cow of 0.375% coumaphos, 0.2% Bayer 9017, 0.5% Hooker 1422, and 0.5% methoxychlor provided effective control of horn flies for 10 days. The lowest test concentrations of 0.06% coumaphos, 0.06% trichlorfon, 0.05% Bayer 9017 and 0.1% fenthion were effective for 6 to 8 days or about as long as 2- to 4-times higher concentrations. In comparative tests back rubbers treated with 0.25, 0.5, and 1.0% Bayer 9017; 0.5 and 1.0% coumaphos; and 0.5% ronnel maintained effective control of horn flies for 7 to 8 weeks, whereas these treated with 0.5% dimethoate and 0.5% Famophos were effective only 4 weeks. Observations suggested that loss of effectiveness was due to loss of the oil solvent since the odor of insecticide was still detectable. Retreatment of each backrubber with 1 gallon of oil resulted in 4 weeks additional control, thus confirming the above observation.

In Mississippi, a series of tests were runto compare the effectiveness of 0.5% oil solutions of 10 insecticides applied in low volumes by automatic sprayers. Single applications of ronnel, Ciodrin, dioxathion, Bayer 9017, Shell Compound 4072 and Dowco 175 provided excellent control of horn flies for 6.5 to 8.5 days. Other materials were equal or slightly less effective than toxaphene (standard) which gave satisfactory control for 5 days.

6. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Of twenty new compounds screened for larvicidal effectiveness at 10, 1.0, and 0.1 ppm in screw-worm larval medium, four were highly effective killing all the larvae at 1.0 ppm: Namely, Shell SD-8964, Shell SD-8988, Shell SD-8967, and Geigy GS-12968. None of the compounds screened were effective at 0.1 ppm.

In field tests in Mexico, cattle infested with 1- and 2-day-old screw-worm larvae were sprayed or dipped in promising insecticides. Shell Compound 4072 in a dip or spray at 0.1% killed all the larvae, as did CELA S-1942 in a 1.0% spray. Hooker HRS-1422 as a 0.25% spray and Shell Compound 4072 as a 0.08% in a dip were fairly effective but permitted a few larvae to survive.

Previous research has shown that sprays containing 0.1% or higher concentrations and a dip containing 0.1% of Shell Compound 4072 are effective screw-worm larvicides. In new tests in Mexico, cattle with wounds containing 1- and 2-day-old screw-worm larvae were dipped in vats containing either 0.05% or 0.1% Shell Compound 4072. At examination 24 hours after treatment, no live larvae were found in wounds on cattle dipped in 0.1%. All 1-day-old larvae were killed by 0.05%, and live 2-day-old larvae were found in only 1 of 16 wounds.

Telodrin as a 0.05% spray killed both 1- and 2-day-old screw-worm larvae but it also killed 3 of the 4 cattle treated. Bayer 37289 (0.25% spray) and Bayer 38333 (0.1% spray) killed all the larvae in one test, but not in another. Sprays containing 0.1% of ethion, 0.1% of Dowco 175, 0.05% of demetilan, 0.01% of Bayer 29952, 0.01% of Stauffer N-2790, or 0.01% of Bayer 38156 were ineffective.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas 113 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Ten materials showed systemic activity in one or both types of administration. The most effective materials, dosages, and routes of administration were as follows: Shell SD-9129, 5 mg/kg, O and SC; Spencer S-6900, 25 mg/kg, O and SC; and CELA S-2225, 25 mg/kg, O. Seven other materials were effective at dosages of 50 to 200 mg/kg.

In Texas field tests were conducted on small numbers of Government-owned cattle (2 to 4) to evaluate the effectiveness of a number of materials that had shown promise in screening tests and of several older effective materials administered in different ways at several dosages. Materials giving 91-100% control of grubs when administered in the feed for 10 days were as follows: Bayer 37341 and Bayer 37342, 1.0 mg/kg; and Famophos, menazon, and Vamidoate, 5.0 mg/kg. As drenches, CELA S-1942 at 100 mg/kg and Shell SD-8949 at 50 mg/kg gave 91 and 100% control, respectively. Menazon as a 1.0% spray gave 100% control. Other materials were ineffective.

In Texas field tests were conducted on cooperative cattle on 9 ranches with several experimental materials and with a number of older systemics administered at different dosages in several types of formulations. In pour-on tests materials, concentrations, formulations, and the lowest rates of application that gave 95-100% control were as follows: 4.0% oil suspension of coumaphos, 10 mg/kg; 15.5% water emulsion of Bayer 37342, 100 mg/kg; 4.0% water emulsion and oil suspension of Ruelene, 25 mg/kg; 7.75% water solution of trichlorfon, 50 mg/kg; and 10.2% oil suspension of ronnel, 150 mg/kg. Conventional spray treatments giving 92-100% control of grubs were 0.25% fenthion emulsion, 0.5% Rulene water suspension, 70 mg/kg; 0.25% Shell Compound 4072 water suspension, and 1.5% trichlorfon water solution, 250 mg/kg.

In Texas field tests were conducted to evaluate the effectiveness of 6 materials on Wyoming cattle infested with the northern cattle grub, Hypoderma bovis, as well as the common cattle grub, H. lineatum. Oral administration of Stauffer R-3828 at 25 mg/kg gave 90% control of grubs. The other materials were partially or completely ineffective.

In Oregon extensive field tests were conducted to evaluate the effectiveness of 7 insecticides as pour-ons and 2 as sprays for the control of cattle grubs. In these tests, pour-ons of 8% Ruelene in water emulsions at 52 mg/kg and in oil solutions at 25, 34, and 46 mg/kg per animal gave 99% control of grubs. Similar results were obtained with pour-ons of 8 and 12% trichlorfon at 30 and 45 mg/kg, and 2% fenthion at 7.5 mg/kg. Pour-ons of ENT 25482 at 91 mg/kg and Shell SD-8436 at 12 mg/kg gave 97% control of grubs and Shell SD-8447 at 121 mg/kg was only slightly less effective. Sprays of 0.1 and 0.25% Imidan® showed 88-89% control but lower concentrations were ineffective.

8. Horse Flies and Deer Flies. In Mississippi, sprays of 2% Ciodrin gave excellent immediate protection of cattle from horse flies but no effect was apparent after 3 hours. Applications fo 1% Ciodrin plus 0.25% dichlorvos provided excellent protection for 7 hours and up to 24 hours in some tests.

9. Ticks. Studies were continued in Texas to develop effective systemics and conventional insecticides for use in the control of several species of ticks on cattle and other animals. A total of 114 new compounds were

screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larval lone star ticks. Only 6 of the materials showed systemic effectiveness. The outstanding materials, dosage and method of administration were as follows: Velsicol FCS-13, 25 mg/kg O and 10 mg/kg SC; and Spencer S-6900, 25 mg/kg O and SC. The other 4 materials were effective at dosages of 50-100 mg/kg by one or both methods of administration.

In Texas 160 insecticides screened in dipping tests against engorged Boophilus females to determine their effectiveness in preventing oviposition and/or hatch of eggs. A total of 44 of the insecticides were ineffective at the highest test concentration of 1.0%. The remainder were effective at 1.0% or lower concentrations. Materials that were effective at the lowest test concentration of 0.01% in preventing oviposition were as follows: Shell SD-8448 and SD-9102, and Niagara NIA-9227. Materials which permitted light oviposition but prevented hatching of eggs were as follows: carbophenothion, Stauffer R-2964, N-3727, and N-3794, Monsanto CP-40272, and Wm. Cooper 57-H-62.

Extensive field tests were conducted in Mexico to evaluate the effectiveness of 12 promising insecticides as sprays and/or dips for the control of Boophilus ticks on cattle. In dipping vat tests, concentrations of 0.05-0.1% of Shell Compound 4072 gave 100% mortality of flat and engorging stages and no live ticks were noted after 1 week, indicating that residual material killed all molting stages. Similar results were obtained with sprays of 0.1% Shell Compound 4072 and Dowco-175 and 0.01% Bayer 38333. Sprays of 0.25% Hooker HRS-1422, 0.1% ethion, 1.0% CELA S-1942, 0.25% Bayer 37289, and 0.01% Bayer 38156 were highly effective but a few ticks were still alive on treated animals after 1 week. Sprays of two materials--0.05% Teleodrin and 0.01% Bayer 29952--killed or severely poisoned cattle. Dimetilan and Stauffer N-2790 were not highly effective.

In field tests in Texas, sprays of 0.1% Shell Compound 4072 and fenthion, 0.25% Imidan and 0.5% CELA S-1942, malathion and toxaphene gave highly effective control of the winter tick, Dermacentor albipictus, on cattle. Little or no reinfestations developed on treated animals within 1 month after spraying.

In field tests in Texas, sprays of 0.25% coumaphos and Imidan, 0.03% diazinon 0.1% Shell Compound 4072, 0.3% Ciodrin, 1.0% trichlorfon, and 0.5% toxaphene gave excellent immediate control of lone star ticks on cattle. Four other materials failed to give satisfactory control. Pour-on applications of 8.0% trichlorfon and 2.0% Hercules 7522, which are excellent systemic treatments against cattle grubs, were relatively ineffective systemically against ticks.

Extensive field tests were conducted in Texas to evaluate the effectiveness of 32 insecticides as sprays and/or dusts for the control of spinose ear tick, Otobius megnini, in the ears of cattle. All of the materials except

Dri-Die and Dowco-175 dusts, and menazon sprays gave excellent to complete control of infestations of the spinose ear tick. However, the only treatments still showing effective control after 1 month were 5% dusts of coumaphos and Shell Compound 4072, 1% Hercules 7522 dust and 0.3% Ciodrin spray.

Small-scale field tests were conducted in Florida to evaluate several insecticides for effectiveness against the tropical horse tick (Dermacentor nitens), the vector of equine piroplasmosis. In these tests dermal and ear applications of 1% lindane, 0.3% Ciodrin, 0.25% Imidan and coumaphos, and 0.1% Shell Compound 4072 gave 100% immediate control of ticks but light reinfestations developed in all instances in 2 weeks. Dri-Die dust was ineffective. In systemic tests trichlorfon at 10 mg/kg a day for 10 days was completely effective in clearing ticks from the ears of horses. Fenthion at 5 mg/kg for 5 days, Famophos at 10 mg/kg for 4 days and Hercules 7522 at 5 mg/kg for 4 days reduced but did not completely eliminate tick infestations.

Surveys in southern Florida showed D. nitens to be present at 4 of 15 locations examined but populations were high in only one. Insecticidal treatment of pastures and horses apparently have eradicated the tick in the other 11 locations.

10. Lice. In Mississippi 14 promising insecticides were evaluated by the spot test method against cattle lice. Three materials, Stauffer B-10046, R-5724, and R-5725 gave 100% immediate kills of motile lice but all permitted reinfestations to develop in 14 days. In field tests, two applications of 5% dusts of coumaphos, carbaryl and dioxathion 2 weeks apart eliminated lice on cattle. Similar applications of 5% methoxychlor gave excellent control but did not completely eliminate the lice.

In Nebraska treatments of groups of cattle with 0.5% ronnel applied with a Bean Rotomist sprayer eliminated all motile stages of cattle lice. However, after 1 month light infestations were again present on some animals.

C. Insecticide Residue Determinations

1. Residue Studies. In Texas tests were conducted to determine the levels of residues in tissues of cattle forced to use back rubbers treated with 1 and 2% ronnel-oil solutions four times daily for 28 days. Small average residues of 0.005 to 0.05 ppm were found in the fat after 2 weeks but only negligible amounts were present after 4 weeks and none whatever could be detected 2 weeks after treatments were discontinued. Residues in muscle, liver, kidney, heart, brain, and spleen were barely detectable after 2 weeks treatment and none whatever could be demonstrated thereafter.

Additional studies were conducted to determine the distribution of P^{32} ronnel dermally on cattle forced to use backrubbers treated with this material. Comparisons were made between animals receiving 2 and 4 exposures per day for 4 weeks. Distribution as indicated by analyses of hair samples was very irregular but most of the insecticide was concentrated along and adjacent to the central back line and tip of the head. Cattle treated 4 times a day received about twice as much insecticide as those treated 2 times daily. No ronnel was present on the hair 2 weeks after the last treatments.

In Texas two tests were conducted to determine the sites of accumulation and amounts of residues in various tissues of cattle resulting from dermal sprays of 0.1% Shell Compound 4072 emulsion. In one test, the cattle were sprayed weekly for 12 weeks; in the other test they were sprayed 6 times at 2-week intervals. In the 12-weekly spray tests, analyses 1 week after the first spray showed residues in the fat ranging from 0.007 to 0.045 ppm (average 0.02 ppm). Residues increased slightly with successive weekly sprayings to a peak average of 0.14 ppm (range of 0.097 to 0.196 ppm) after the eighth spraying. One week after the twentieth and last spraying, residues in the fat averaged only 0.01 ppm (range 0.008 to 0.016 ppm). No residues were detectable 2 weeks after the last spraying. In the biweekly spray test analyses 2 weeks after the first and third sprays showed only 0.005 ppm in the fat but residues increased somewhat after the fifth and sixth sprays, averaging 0.117 and 0.133 ppm, respectively. Additional analyses of fat from animals slaughtered 2 weeks after the sixth and last spraying showed average residues of 0.065 and 0.112 ppm in renal and omental fat, respectively. No residues were detectable 4 weeks after the last spraying.

Analyses of tissues from a calf slaughtered 7 weeks after being sprayed with 0.25% Imidan showed no residues in samples of fat, muscle, heart, liver or spleen.

In Maryland, analyses were made to determine the levels of residues in fat samples of swine 1, 4, 7, 14, 21, and 28 days after treatment with 0.05% lindane emulsion. Maximum residues were indicated 4 to 7 days after treatment but declined steadily thereafter. Four weeks after treatment only 0.1-0.3 ppm was present in the swine fat.

In Maryland, as a result of the detection of residues of heptachlor epoxide in the fat of cattle slaughtered at the Agricultural Research Center at Beltsville, analyses were made to determine if residues were present in the milk of three experimental dairy herds. The over-all average residue of heptachlor epoxide in the milk was below 0.01ppm. Analyses of eight lots of hay and one lot of alfalfa pellets which were being fed to the cattle showed residues ranging from 0.00 to 0.048 ppm of heptachlor epoxide. Subsequent tests did not show detectable residues in the feed but low levels of heptachlor epoxide persisted in the milk of selected animals from experimental herds.

2. Toxicity Studies. Research was continued in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals to livestock.

Studies were conducted to determine the normal patterns of certain enzymes in average cattle as a prerequisite for studying the effects of chemical poisoning on enzyme patterns and the effects of oxime-type cholinesterase reactivators (2-PAM, DAM and TMB-4). Cattle poisoned by Dioxathion caused elevations in activity of serum glutamic oxalate, pyruvate transminases, alkaline phosphatase, and blood beta lipoprotein but these increases were minimized by the administration of 2-PAM and TMB-4. All three test oximes prevented decreases in gamma globulin. These results indicated that TMB-4 was slightly more beneficial than 2-PAM. DAM did not appear beneficial at the levels (10-20 mg/kg) tested.

Cattle were poisoned with an oral dosage of dichlorvos to determine the effects on serum glutamic oxalacetic and pyruvic transminase, aldolase and alkaline phosphatase. Oximes were given some of the cattle to determine their protection of those enzyme systemics. DAM and 2-PAM kept the enzyme activities of the mildly poisoned animals near normal, whereas TMB-4 appeared to cause an increase in activity above normal during the test. From the biochemical standpoint, it appeared that 2-PAM and DAM offered more protection to the enzyme activities than does TMB-4.

Cattle were poisoned with coumaphos and enzyme systems studied in serum. Some of the cattle received antidotal therapy with 2-PAM. Glutamic dehydrogenase, sorbital dehydrogenase, phosphohexose isomerase and serum arginase were studied in an effort to find significant enzyme activity alternations indicative of possible tissue change. No significant differences were noticed in the enzyme activities regardless of treatment. Mortality was reduced by 2-PAM in coumaphos poisoned animals, but this benefit could not be detected in the enzyme studies.

Additional studies were conducted to determine the effectiveness of these three oximes in reversing cholinesterase inhibition induced by organic phosphorus compounds. Each of the compounds was useful, but 2-PAM and TMB-4 appeared to be superior to DAM. Particularly encouraging was the beneficial effect of these compounds in cattle poisoned by coumaphos; usually such animals do not readily respond to atropine, the pharmacologic antidote. Oximes combined with atropine markedly increased the number and speed of recoveries.

Studies were conducted to determine the effects of the chemosterilant, apholate, when fed to Jersey cattle at 1 mg/kg daily throughout one gastation period. A deficiency of white blood cells appeared in one heifer after 80 daily doses and in the others after 110. One heifer died after 335 doses, one delivered a calf and died after 531 doses, one delivered prematurely and survived 581 doses, and the fourth delivered at term and survived 629 doses. In additional studies the new chemosterilant, hempa, appeared to be

considerably less toxic than apholate, tepa and metepa, but it produced the same deficiency in white blood cells.

Studies were conducted to determine the toxicity of 72 insecticides to cattle, sheep and goats. Toxicities ranged from impossibly dangerous to reasonably safe. Promising new insecticides showing low toxicities were Shell SD-8447 and CELA S-1942.

D. Biological Control

1. Mosquitoes. At Lake Charles, La., research on pathogens of mosquitoes has been conducted. Field collections throughout the area have shown infections of microsporidia in thirteen species including the genera, Culex, Aedes, Culiseta, Anopheles, and Orthopodomyia. Aedes grossbechi and Orthopodomyia signifera were new host records for a microsporidian. Spore sizes were determined and classification of the microsporidia studied. Transovarial transmission of microsporidian infections was studied in 12 species of mosquitoes and demonstrated in seven of the species.

The fungus Coelomomyces was found in field collections infecting larvae of Culex restuans, C. salinarius, Aedes vexans, A. sollicitans and Culiseta inornata. Culex salinarius, C. restuans and Aedes sollicitans represent New host records for Coelomomyces. Field infection levels varied from very low to over 50%.

A polyhedral virus was reported from larvae of Aedes vexans and Psorophora ferox. Both species were previously unreported as hosts of viruses. Infection levels in the field were very low. A very lethal bacteria was collected from larvae of six mosquito species.

At Gainesville, Fla., two species of Thelohania were found in A. quadrimaculatus, one infecting the adipose tissue and the other the oenocytes. One species was found infecting the oenocytes of A. crucians larvae.

2. Face Fly. Studies in 1963-64 by personnel of the European Parasite Laboratory, Insect Identification and Parasite Introduction Research Branch, in France showed that the adults of Aelochara tristis, a staphylinid beetle, were predaceous on larvae of the face fly and other Diptera breeding in cattle droppings and that newly hatched larvae parasitize face fly pupae. Shipments of this species were received at Lincoln, Nebr., in January and February 1965 and immediate steps were taken to establish colonies. The adult beetles were caged under room conditions (80-85° F; 50-60% RH) and provided fresh cattle manure containing face fly eggs and small larvae. The beetles survived well, oviposited and newly hatched larvae were noted in 12 days. Face fly pupae were made available to the small larvae. Observations showed that the larvae quickly penetrated the face fly puparia and closed the entrance hole. The larvae consumed the face fly pupae and pupated within the puparia. Adult beetles emerged from the puparia through the area

normally used by emerging flies. The combined larvae and pupal period was about 17 days and the adult preoviposition period of about 12 days. Large numbers of adults will be produced and released at selected field sites near Lincoln and studies made to determine the effectiveness of this parasite in reducing face fly populations.

3. Horse Flies and Deer Flies. In Mississippi, large numbers of tabanid larvae were collected periodically and transported to the Kerrville, Texas, laboratory to determine the number and species parasitized and the identity of the parasites. A total of 206 larvae, representing 4 species, were collected in October 1964. Only 3 larvae of T. atratus showed microsporidian infections. In subsequent collections in November 1964 and January 1965, microsporidian infections were found in some of 3 species of tabanid larvae, namely, T. sulcifrans, T. vittiger schwartdi, and T. atratus. The microsporidia in sulcifrans could not be identified but those infecting vittiger schwardti were of the genus Plistophora. Those infecting atratus were Thelohania sp. Healthy atratus larvae readily became infected when fed spors of Thelohania but not when fed those of Plistophora. Microsporidian infections could not be induced in fed or starved healthy larvae held at 10° C. At 25° C, fed larvae readily developed infections but not starved larvae.

In Texas, approximately 300 tabanid larvae were collected and examined during the year and all were free of microsporidia and other parasitic infections.

Studies were conducted to determine the host range of microsporidia (Thelohania sp.) by feeding artificially infected C. macellaria larvae to different species of tabanid larvae. The normal host of this parasite, Tabanus atratus, readily became infected. Of 5 other species used, only one, T. americanus, developed typical spore infections.

Similar studies were made with another microsporidian (Plistophora) but results were negative.

In Texas efforts to develop a reliable spore agglutination test based on sedimentation patterns formed by spores mixed and allowed to settle with rabbit antisera were unsuccessful. In other tests antimicrosporidia spore-rabbit serum reacted positively by agglutination against homologous antigen but further tests must be conducted to ascertain the specificity of the reaction.

4. Ticks. In Texas laboratory tests were conducted to determine the effectiveness of a dust preparation of the fungus Beauveria bassiana against 4 species of ticks. Adults and nymphs of Amblyomma americanum, adults of A. maculatum and adults of Dermacentor variabilis were highly susceptible to the fungus with 90 to 100% kills occurring in 7 to 10 days. Nymphs of the spinose ear tick, Otobius magnini, were not affected.

E. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., studies were continued on factors affecting the attraction of mosquitoes to their hosts and factors affecting the protection time from mosquito bites afforded by repellents. A large olfactometer was developed to study these factors as well as evaluate the efficacy of various attractant materials or factors.

The effort to develop effective space and systemic repellents was continued and slightly expanded. To date several materials show some space repellency to mosquitoes in that they prevent mosquitoes from penetrating 4-mesh screening. Materials exhibiting some systemic repellency were found and further tests will be made on these materials.

Studies were continued at Gainesville to evaluate materials as chemosterilants for mosquitoes and to evaluate the sterility principle of mosquito control. Tests with hempa indicated it would have little value as a residual sterilant for mosquitoes. Further selection and studies were conducted with the apholate-resistant colony of Aedes aegypti to clearly define the degree of resistance. This colony is at least 10 times as resistant to the sterilizing effects of apholate as the unselected, parent colony. Selections to increase resistance will be continued.

A sterile-male release study of Anopheles quadrimcaulatus was made in a semi-isolated area. This site was made more favorable by increasing the number of breeding sites and introducting additional wild stock of this mosquito to populate the area. When sterile males of wild stock were released in this area, sterility of the natural population increased from a very low degree up to 42%. When the releases were changed to sterile colony males the sterility in the natural population decreased confirming earlier work showing behavior differences between colony and wild strains in seeking out wild females. Apparently a sufficient number of males was not released to reduce the population levels of A. quadrimaculatus.

Studies were continued on the evaluation of chemosterilants for mosquitoes at Corvallis, Oreg. The chemosterilant, hempa, was not highly effective in sterilizing Culex tarsalis larvae in that rates as high as 200 ppm were required. As a residual treatment in glass jars, 10 mg of hempa per square foot sterilized adult males completely, but adult females only partially. In wind tunnel tests against adults, a concentration of 10% caused high sterility, whereas a 5% spray caused only partial sterility and non was caused at 1% or lower. Males were generally more susceptible than the females.

At Corvallis studies were continued on sex and ovipositional attractants for mosquitoes. Preliminary tests indicated the presence of a sex attractant in Culex quinquefasciatus, but further tests did not confirm its presence, nor the presence of a sex attractant in Culex tarsalis. Many mosquitoes are known to choose specific types of water for oviposition. Studies have shown

that odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of Culex quinquefasciatus. These odors were not attractive to females of C. tarsalis. However, log pond water itself was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of C. quinquefasciatus but not to those of C. tarsalis. Gravid females of both species were more attracted to distilled water treated with 25 ppm of furfural than to water treated with 5 or 50 ppm.

2. House Flies. At Gainesville, Fla., research was continued on the development of chemosterilants and the sterility principle of control for house flies. Several hundred new candidate compounds were evaluted in primary screening and secondary development tests and many were found to exhibit sterilizing efficacy against both males and females. Particular attention was paid to evaluating two compounds--hempa and hemel--as sterilants by several routes of administration. These two materials will sterilize both sexes of the house fly.

Two series of field tests were conducted at farms in Florida to evaluate the effectiveness of two chemosterilants, hempa and apholate, for the control of house flies. At the farm treated with hempa, house flies were reduced in abundance from 71 per grid to 0 within eight weeks. Grid counts remained at zero for the remainder of the test period. At the farm treated with apholate population levels decreased from 200 per grid to less than 10 within 6 weeks and remained constant at a low level throughout the remainder of the test.

Ninety chemicals were screened as chemosterilants against adult house flies. Six compounds were toxic and 10 reduced the fertility to some extent in fly food or sugar. Thirty-two compounds previously shown to sterilize house flies were also tested again at higher or lower concentrations.

Tests were conducted with 21 compounds to determine their effectiveness as male house fly sterilants. Of the 14 chemicals Olin 53330, Squibb Olin 53331, Squibb Olin 53356, and Squibb Olin 53263 sterilized at a concentration of 0.05% in the sugar diet. Sankyo Co. RES-101 induced sterility at this dosage in sugar in the first two eggings.

Basic studies were continued on the cytological effects of chemosterilants on house fly reproductive systems and previous sectioning, fixing, and staining techniques have been used to study several new chemosterilants.

Olfactometers designed by Gouck and Schreck were used to initiate a search for more effective house fly attractants. Nineteen compounds were tested as house fly attractants, using Edamin as a standard. Beef protein concentrate was attractive to females but not to males. The other materials were not as effective as Edamin.

At Corvallis, Oreg., research was conducted on chemosterilants and attractants for the house fly and the little house fly. With the little house fly hempa caused sterility as a residual deposit on glass at 50 mg/ft^2. However, the sterilizing dose caused some fly mortality. Higher doses were highly toxic to the adult flies; lower doses did not sterilize. When fed orally in the adult food to the little house fly, hempa was toxic at 0.25% and lethal at 1.0%. High, but incomplete sterility was caused at concentrations as low as 0.01%. Topical treatments of hempa and hemel sterilized males of the little house fly without causing mortality, but not the females. In general treatments causing a high degree of sterility did not affect the mating competitiveness of females. Four known antioxidants exhibited little effect on egg production or egg hatch with the little house fly. Dosages of gamma radiation greater than 1000 r given to pupae of the little house fly prevented oviposition by emerging adults.

At Corvallis research was continued on the sex pheromone in house flies. The presence of a low titre of pheromone in extracts of pupae and young females was shown and confirmed. Higher activity of the pheromone was demonstrated in 3-day-old flies. The presence of the pheromone was shown in female flies from strains of different origin. Males of different strains reacted to extracts from females of different strains, though differences in behavior were apparent.

At Corvallis, Oreg., further studies showed that the sex pheromone in female house flies increased with the age of the flies, with the greatest increase occurring on about the third day after emergence. Comparative tests with benzene extracts of female house flies showed that high concentrations applied to pseudo flies inhibited male response. Similar inhibition occurred when normal extracts were applied to large surfaces within test chambers. Comparative tests with extracts of females with different solvents showed a much greater amount of the pheromone in hexane than in other solvent extracts. Efforts are being made to determine the chemical nature of the pheromone.

At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of house flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

3. Stable Fly. At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of stable flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or

screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

4. Face Fly. At Beltsville, Md., additional tests of the responses of 3-day-old female face flies to monochromatic light confirmed that blacklight is highly attractive under conditions of confinement and that wavelengths in the red and yellow spectral regions are unattractive. Refinements of the testing procedure and methods of data analysis are being made in an attempt to more clearly establish differences in attractiveness.

5. Horn Fly. In Texas, topical applications of 1 µg/fly of apholate sterilized adult horn flies. Lower dosages of 0.5, 0.2 and 0.1 µg/fly reduced the hatch of eggs but did not confer complete sterility. Males were more easily sterilized than females. In feeding tests, flies feeding overnight on a diet containing as little as 0.01% apholate were completely sterilized.

6. Screw-worm. In Texas 20 of 255 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worms when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration, some sterilized only one sex, and some sterilized both male and female flies. A review of past chemosterilant screening revealed that of the aziridine compounds screened, 92 were effective either by multiple-oral administration or topical application, or both. Only 8 were less than 100% effective when administered orally. In the future, routine use of topical applications in chemosterilant screening will be dropped in favor of multiple-oral administration.

It has been shown that there is a differential susceptibility between males and females sterilized with metepa. Tests with uredepa (ENT-50450) showed similar results, with the males about 9 times more susceptible than females on the basis of dosage/unit of body weight. There was also a greater variation in results obtained with females than with males. Starvation also increased the effectiveness of uredepa. The antifertility effects of another chemosterilant, ENT-25296, were enhanced by subjecting treated flies to periods of temperature stress (98° or 14° F) after administration of sterilizing or highly effective substerilizing dosages.

Male and female screw-worm flies can be sterilized by exposure to certain chemosterilants, but most of these chemicals adversely affect mating activity, longevity, or vigor. Three new chemosterilants were found that equal or surpass radiation in their effectiveness in achieving sterility of screw-worms. ENT-50838 applied topically provided a wide margin of safety between the minimum toxic quantity and the sterilizing dosage. Males sterilized with this material were hypercompetitive; they were sexually more aggressive than irradiated flies. The other two compounds, ENT-50716 and ENT-50781, were more toxic to the flies, but they were approximately equal to radiation as sterilizing agents.

In Texas, 154 chemicals and other materials were screened as screw-worm attractants. Of these, 22 were equal to or better than the standard liver bait and require further evaluation. Some have been tested in the field in limited tests. The most outstanding were isovaleraldehyde, ethyl isovalerate, and an ethanol extract of the flowers of Yucca treculeana. Liver-baited traps were seldom as effective as traps containing these materials. The presence of blooming wild flowers interfered with these tests; tests made during peak blooming seasons usually gave negative results.

The presence of a pheromone produced by males that is attractive to virgin female screw-worm flies was confirmed. In Texas, by means of the cold-trap method, 3500 ml of condensate were collected over a 5 1/2-month period from a cage containing virgin male screw-worm flies. Benzene and chloroform extracts of the condensate were capable of changing the behavioral pattern of virgin female flies. Sexually mature females in the presence of the odor go through "searching" motions and finally behave as in an aggressive mating "strike." Young (1-2 days old) females gave little or no response to the male odor, but 3-day-old females exhibited a definite activity, including the imitation of "male-type strike." Four-day-old females reverted to the response of 1-day-old females, but activity increased again in 5-day-old females. The greatest response was observed in 6-day-old females, with 7- and 8-day-old females showing a decrease in total number of "strikes." This decrease may have been due to wing damage, normal at this age. Other studies are in progress, including fractionation of the extracts to find the effective material. Another extract, made by filtering the air in the eradication colony room, brought a response on the part of both males and females.

7. Ticks. In Texas, studies on the effects of radiation on lone star ticks showed that dosages of 250 and 500 r did not affect fertility of adults treated 1 week after molting from nymphs. However, a dose of 500 r sterilized females 1 day after engorging. Doses of 250 and 500 r had no effect on engorging and molting of nymphs or on fertility of resulting adults. However, at 1000 r the percentage molting of nymphs was reduced and resulting adults did not engorge or lay viable eggs.

F. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas and Mississippi in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the State Experiment Stations to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. Texas, monthly surveys were made to determine the identity and abundance of external parasites on infected and clean (segregated) herds of

cattle. Lone star tick populations were light on cattle in January, increased gradually during February, March and April, were heavy from May through July and then declined rapidly to insignificant numbers by September. Winter ticks first appeared late in October, increased gradually to peak abundance in late December, then declined to insignificance by March. The spinose ear tick was present throughout the year, with populations being moderate to high at practically every examination. Cattle lice populations were extremely low throughout the year. Horn flies appeared in April, increased steadily to moderate numbers by June, remained at this level until late September, and disappeared with cool weather during late October and early November. Light to moderate grub infestations were present in the backs of cattle from early January until mid-March. Some transmission continued to occur in the infected herd but the segregated herd remained clean. It was thus demonstrated that young calves removed from the presence of carrier cows could be maintained anaplasmosis-free. The test was discontinued this year.

In Mississippi, studies on the relative importance of day- and night-feeding insects in the transmission of anaplasmosis, which were initiated in 1963, were continued in 1964. In the main test, three groups containing negative, splenectomized steers were used, one group being exposed continuously, one during the day only, and one at night only. The test period of 6 weeks (June 1 - July 13) was divided into three 2-week intervals and during each interval one other group of cattle of the same composition was exposed continuously in hopes of pinpointing the 2-week period in which most transmission occurred. Each group was exposed daily with animals infested with anaplasmosis.

During the first two weeks (June 1-13) horse flies were moderately abundant, but declined in numbers abruptly and only small numbers were present the last 3 weeks of the test. Populations of horn flies were relatively low and stable flies relatively high throughout the test period. Mosquitoes were fairly numerous the first week of the test but populations declined thereafter and remained low throughout June. Populations rose rapidly early in July and were high during the last two weeks of the test. The mosquito population was largely Psorophora confinnis until the last week of the test, when Anopheles quadrimaculatus increased abruptly to make up about 40% of the population.

In the main 6-weeks test, two cases of anaplasmosis developed in each of the continuous- and day-exposure groups, but none occurred in the night-exposure group. In the other groups exposed continuously for 2 weeks, one case each occurred in the first (June 1-16) and second (June 16-29) exposure periods but none developed in the third (June 29-July 13). These results indicate that anaplasmosis transmission most likely occurred during June when horse flies were most abundant. This indication is supported by the fact that no transmission occurred in the cattle exposed at night when only mosquitoes attacked them. However, additional studies are needed to clarify

the role of mosquitoes as possible vectors and to determine whether one or more species of horse flies is capable of transmitting the disease.

At Beltsville, Md., studies on the transmission of bovine anaplasmosis and the development of the disease organism in experimental vectors were continued in cooperation with personnel of the Animal Disease and Parasite Research Division.

Efforts to colonize a "Nevada line" of Dermacentor andersoni were unsuccessful. The larval progeny from a single engorged female received from Nevada did not attach and feed on the test calf, and all died.

An adult-to-larva hereditary transmission trial with Dermacentor occidentalis was negative. Several thousand larvae attached and fed on the test calf, but anaplasmosis was not transmitted. The calf was proved susceptible by inoculation with Anaplasma infected blood.

Studies were conducted with Dermacentor occidentalis to determine whether or not the ticks can become adapted to infection with Anaplasma marginale by feeding one or two of the developmental stages in each generation on calves acutely infected with A. marginale. The plan of study was to feed adults on infected calves, feed the resulting larval progeny on guinea pigs and then test part of the ensuing nymphs on a susceptible calf. If transmission did not occur, the remaining nymphs and suceeding adults were to be fed on acutely infected calves and tested again in the following generation. In the first generation studies, the adult-to-nymph trial was negative and the test calf proved susceptible by challenge. Approximately 1,000 nymphs were allowed to feed on an infected calf and 50 of the resulting adults were tested on a susceptible calf for a trans-stadial transmission. This experiment has not yet been concluded. The remainder of the D. occidentalis adults, infected as nymphs will be fed again on an infected calf and tested again as 2nd generation nymphs.

Systematic studies on Anaplasma-infected and non-infected D. occidentalis salivary glands are being conducted concurrently with each transmission experiment.

2. Equine Piroplasmosis. During the last year at Beltsville, Md., larval progeny of field collected D. nitens adults from two Florida sources were tested on susceptible horses to determine their infectivity status. Transmission did not occur and subsequently both test horses were shown to be susceptible by inoculations of Babesia caballi infected blood.

A transmission trial with the larval progeny of D. nitens adults collected in Puerto Rico was also conducted with negative results. This test horse was also subsequently shown to be susceptible by inoculation of Babesia caballi infected blood.

Transmission experiments are now under way with the larval progeny of D. nitens adults collected from infected horses in Florida during June 1965. Results of these tests are not yet available.

Cytological studies on D. nitens were conducted to gain a better understanding of the disease agent and its relationship to the vector. All stages of D. nitens were taken from horses infected with Babesia caballi and prepared for histological study. Structures believed to be developing forms of Babesia caballi were observed in sections of some specimens.

Another and more virulent strain of Piroplasmosis, Babesia equi, was found for the first time in Florida this year. The vector of this disease has not been determined as yet but transmission studies with several species of ticks and other external parasites are planned.

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Insect Vectors of Diseases

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AREA NO. 14. DAIRY CATTLE INSECTS

Problem. Flies, mosquitoes, grubs, lice, and ticks are common pests of dairy cattle that cause important losses in all parts of the United States. Heavy attacks by biting flies lower milk production by 5 to 20%. Total losses to dairy cattle attributable to insects and ticks are estimated to exceed $200 million annually. Certain insect pests are also involved in the transmission of diseases of dairy cattle. Methods of control for dairy insects have received setbacks during recent years because the best available insecticides and most promising new materials produce residues in milk. In addition, house flies around dairy establishments have developed resistance to DDT and other insecticides. There is, therefore, great need to find safe, effective, non-residue insecticides and repellents to control these insects and ticks. Effective systemic insecticides and ways of administration which would avoid residues are needed to combat grubs in dairy cattle and to prevent the face fly and horn fly from breeding in the manure. New approches to control, including radiation and chemosterilants, need to be explored further to determine their feasibility for the control of several dairy-cattle pests. Research should be continued to support the Southwestern screw-worm eradication campaign. Efforts also should be made to find and evaluate insect pathogens, parasites, and predators for controlling certain dairy-cattle pests. Expanded basic studies on the biology and physiology of these pests are needed to find weak links in their life cycle to serve as a basis for the development of more effective and safer methods of control. Research is also urgently needed on the role of insects in the spread of diseases of dairy cattle.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of dairy cattle. Studies are conducted on the biology, physiology, genetics and nutrition of the screw-worm fly, stable fly, horn fly, horse and deer flies, the face fly, mosquitoes, and other pests; on the nature of insect resistance to insecticides; on the mode of action of insecticides and on their absorption, metabolism and excretion by insects; the effects of irradiation and chemosterilants on insects; insect attractants and repellents; and other new approaches to control. Research is directed towards the development of more effective conventional and systemic insecticides and protective treatments for the control of dairy-cattle pests. Studies are conducted to determine the occurrence of insecticide residues in the tissues and the milk of treated animals. Minor attention is given to the development of sanitation and management procedures and to biological control, especially parasites and predators, for controlling the face fly, stable fly, horse fly, and several other pests. Studies are conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions to develop physical and mechanical methods of control, to evaluate traps and devices for estimating

and controlling natural insect populations and improved or special equipment for the application of insecticides to dairy cattle. Limited research is conducted on the role of insects and ticks as vectors of animal diseases, with special emphasis on bovine anaplasmosis. The research is conducted in major laboratories at Kerrville, Tex., Corvallis, Oreg., and Gainesville, Fla., and at satellite stations at Beltsville, Md., Stoneville, Miss., Lincoln, Nebr., and Fresno, Calif. Research is conducted at McNeese State College, the University of Southwestern Louisiana, and the University of California under contracts.

The Federal scientific effort devoted to research in this area totals 16.6 professional man-years. Of this number 6.4 is devoted to basic biology, physiology and nutrition; 3.7 to insecticidal and sanitation control; 2.5 to insecticide residue determinations; 0.3 to biological control; 1.9 to insect sterility, attractants and other new approaches to control; 0.3 to evaluation of equipment for insect detection and control; 0.7 to insect vectors of diseases; and 0.8 to program leadership. The Federal support devoted to research in this area under contracts totals 0.8 man-years, of this number 0.2 is devoted to basic biology, physiology, and nutrition, 0.3 to insecticide and sanitation control and 0.3 to biological control.

PROGRAM OF STATE EXPERIMENT STATIONS

Valuable information on insects affecting dairy cattle is being provided by research in the States. Studies are in progress to determine the abundance, geographical distribution, seasonal variations and economic importance of pest species. Rearing methods are being developed to provide insect specimens (1) for laboratory studies involving the effects of ecological factors on growth and survival; (2) for studying the microorganisms normally present in pest insects; (3) for cattle disease transmission tests performed to determine which insects may serve as vectors; and (4) for control studies.

Various substances are being evaluated for their attractant or repellent effects on such pest insects as flies. Those attractants which exert a significant effect are incorporated as baits with new insecticides or chemosterilants. Various other application methods are also being evaluated.

The development of resistance to insecticides in flies has brought about research to determine methods of combatting it as well as initiating a search for new chemicals. Studies are in progress to determine the effects of repeated heavy insecticide dosages as opposed to light doses, and the influence of fly behavior, development and reproductive capacity on resistance. The mechanism of resistance in the insect in relation to penetration of the integument, distribution, activation, degradation and excretion of the insecticides is also being investigated.

Biological control research is being performed to determine the value of natural agents as supplementary control measures. On dairy cattle, materials

and techniques of application are being tested for their effects on weight gain, milk production, milk contamination and animal health as well as pest control. Milk and tissues are being recovered from treated animals and examined for pesticide content. Detection of metabolites as well as the original compounds is being emphasized.

There are 16.1 professional man-years devoted by the States to research on insects affecting dairy cattle.

PROGRESS-USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies were continued on the biology of Anopheles quadrimaculatus by artifically augmenting the population in an isolated breeding area. It was shown that: (1) the density of the population was increased in the study area when a constant breeding area was supplied to egglaying females and when a blood source was provided, (2) wild females preferred natural ponds as an egg-laying site but used artificial ponds when natural areas were not present, and (3) Anopheles quadrimaculatus adults preferred resting boxes which were painted black and placed on a horizonal plane.

New rearing diets and rearing techniques were evaluated for Anopheles quadrimaculatus. Rye grass infusion or extract in the rearing medium accelerated larval development. There was a positive correlation between the number of larvae per rearing pan and larval mortality. Protozoans were an important source of food for the larvae. A rearing method incorporating all of these factors increased survival and size of the insects and decreased the time required for development and their tolerance to insecticides. Similar results were obtained with four other species of mosquitoes, Aedes aegypti, A. taeniorhynchus, Culex quinquefasciatus, and Aedes triseriatus.

A new method of separating pupae of Anopheles quadrimaculatus from larvae has been adopted. When mixtures of both are placed in ice-water, larvae sink and pupae float allowing rapid separation through the use of a funnel. Time required to separate the stages in colony production was reduced 86%.

At Corvallis, Oreg., studies were continued on the biology of the mosquito, Aedes increpitus, in the Willamette Valley. During the winter months of the last three years, larvae of this species have been collected in numerous habitats of the flood plain of the Willamette River. Following an unusually protracted period of subfreezing temperatures during which a low of 8° F was registered and near-record floods during which all low lying areas in the Willamette Valley were inundated for several days, larvae could be readily collected. The strain of increpitus in the Willamette Valley apparently has become well adapted physiologically to the rigors of the area over a long term period of time. In other studies, a strain of Culex pipiens

quinquefasciatus, which is orange in color as 4th-instar larvae and newly formed pupae, has been isolated. The strain has bred true for 3 generations and appears to be genetically recessive.

At Lake Charles, La., basic studies on the biology of floodwater mosquitoes was conducted. Studies have shown the comparative longevity, blood-feeding, and oviposition patterns of different species of flood-water Aedes species. Aedes taeniorhynchus, A. sollicitans, and A. infirmatus are more important as pest mosquitoes than other species that occur in the area. Studies on the amount of blood ingested by 12 pest mosquito species occurring in Southwestern Louisiana indicated that females of all species ingested sufficient blood at one feeding to at least double their body weight. Psorophora cyanescens, Aedes atlanticus-tormentor, and Anopheles quadrimaculatus, more than tripled their body weight with blood from one feeding.

Research has been conducted under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the production, relative abundance and dispersal of pest mosquitoes in the Gulf Coast area of Louisiana. Data has been maintained on rainfall and temperature in relation to mosquito production and some information has been obtained on the influence of rains versus tidal action in mosquito production. An impoundment is being developed to study the effects of impounding and water management procedures on mosquito production in the area.

2. House Fly. At Gainesville, Fla., research was continued on basic biology of the house fly. Evidence was obtained that an olfactory attractant, or pheromone, specific for the males of Musca domestica L. is not produced only by the females. The attractant was found on contaminated holding cylinders and on dead and non-virgin females. In addition, live males were also somewhat attractive. The degree of attraction was of a low order, resembling other reported sex pheromones of the house fly. This attractant was soluble in methane and slightly soluble in benzene. Data also showed that the time of day at which pupal eclosion occurs is influenced by photoperiod, but photoperiod may not be the only controlling factor. Response to insecticides was also shown to be regulated by photoperiod.

In mating experiments, female house flies mated more readily with males from their own strain than with those of other strains. When normal females from the laboratory or Grand Turk (wild) strains were confined with normal males from one strain and chemosterilized males from the other, they mated more readily with males of their own strain, whether sterilized or not. Chemosterilized males competed more successfully than normal males of the same strain.

At Corvallis, Oreg., research was conducted on the genetics and physiology of house flies and data developed in these studies were used to elucidate mechanisms of insect resistance to insecticides.

Several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. Several of the mutant strains have been defined genetically and are being maintained. Most mutants involved wing form, wing positioning, or pattern of wing venation. Three established mutant strains, classic wing, stubby wing, and dot vein have proved useful in the genetic analysis of insecticide resistance. For example two DDT resistant strains of house flies were found to possess a fifth chromosomal dominant which confers moderate resistance to DDT, but does not confer resistance to o-chloro DDT. In addition, one of the strains possessed a second chromosomal recessive which conferred moderate resistance to DDT and high tolerance to o-chloro DDT. The presence of both factors in a strain confers virtual immunity to DDT. Substrains were isolated, each possessing only one major factor for resistance and the nature of the two independent factors for resistance confirmed through appropriate crosses and bioassays. Resistance associated with the fifth chromosomal dominant is that for DDT dehydrochlorinase. The mechanism of resistance associated with the second chromosomal recessive is unknown, but apparently does not involve dehydrochlorination.

Physiological studies of mechanisms of resistance to organophosphorus insecticides in house flies showed that blocking of Ali-E with a selective inhibitor increased the accumulation of paraoxon and also the toxicity of parathion and paraoxon in both susceptible and parathion-resistant strains. These results indicated that Ali-E is an important detoxifying enzyme. The role of Ali-E in organophosphate poisoning appeared to be related more to detoxication of paraoxon than of parathion in both susceptible and parathion-resistant house flies.

A resistance factor (esterase(s)) for parathion in house flies was shown to be transmitted in a dominant manner and was at least 1000 times less sensitive to inhibition by paraoxon than a corresponding esterase present in a susceptible marker strain.

In other studies successful mating of house flies kept in complete darkness from the time of emergence from pupae to separation of the sexes occurred. Sex ratios were normal among the offspring.

3. Stable Fly. At Gainesville, Fla., studies were continued on the stable or dog fly, a serious pest of animals and humans along the northwest coast of Florida. A general outbreak of stable flies occurred during the last week of August and the first 2 weeks of September in 1964. Population counts around motels and other structures and on beaches ranged from landing rate counts of 2 to 94 flies per man per minute. Bay grass deposits in 1964 were much heavier in 1964 than in 1963. Observations indicated that the insects developed in the bay grass in about the same length of time as was required for development in CSMA medium under laboratory conditions.

Tests indicated that the effectiveness of insecticides against stable flies varied with the time of exposure after blood feeding. Other research in

large outdoor cages indicated that landing rate counts of stable flies were a good index of total populations since the same percentage of flies landed on human observers when the total number of flies was varied.

In Nebraska the exposure of successive generations of stable fly larvae to DDT in the larval medium resulted in a gradual increase in tolerance to the insecticide. By the 39th generation tolerance had increased by 45-fold, but by the 42nd generation tolerance had declined to about 2-fold. The reasons for this rapid decline have not been determined.

In Texas studies were conducted to determine the effects of 4 different conditions of light (continuous light or dark, 12 hours light and 12 dark, and normal daily fluctuation) on the pattern of emergence of adult stable flies from pupae. Emergence in all tests was essentially circadian in pattern, with peaks between 6 and 9 a.m. The bulk of emergence in each group occurred on the 3rd, 4th, and 5th days after first emergence.

In Texas studies were conducted to determine the function of the antennae in feeding and mating behavior of male and female stable flies. When the antennae were completely covered with Plexiglass glue the flies did not feed and mating was greatly reduced. Additional tests showed that blinding the flies by covering the ocelli with black paint also greatly reduced mating. These results indicate that the antennae play an important role in feeding and mating responses of flies and that sight (i.e., light) is necessary for maximum mating response. Further studies showed that when stable fly pupae were held in total darkness, adults emerged and fed but only a small percentage mated and produced viable eggs.

4. Face Fly. In Nebraska, laboratory studies on the reproduction of the face fly showed that as the proportion of males to females was increased, reproduction per female decreased. This was true when only fertile males were mated with the females as well as when various ratios of fertile and sterile males were used. Decreased reproduction apparently was due to harassment by males which reduced female longevity and thereby reduced oviposition.

Studies were conducted under laboratory conditions to determine the egg-laying pattern, number and fertility of eggs and longevity of laboratory reared face flies. Female flies were allowed to mate once then confined individually in small cages for observation. Longevity of the flies ranged from 9 to 60 days, averaging 28.4 days. Batches of eggs per female ranged from 0 to 9 and the number of eggs per female varied from 0 to 181, averaging 56.6. Viability varied from 0 to 89.0%, averaging 60.2%. A few flies oviposited when 4 days old but the average age was 10 days. Results of these tests indicate that multiple matings may be necessary to insure sufficient spermatozoa to insure fertilization of all eggs laid.

Preliminary studies with 3 different strains of face flies showed very little difference in the longevity, fecundity, and rate of development of

the colonies maintained under continuous light and under 16 hours of light and 8 hours of darkness. No circadian rhythms were observed but studies are being continued.

In Nebraska studies were conducted to determine the pattern and distance of dispersal of marked, laboratory reared flies released in the field. Observations indicated that the flies dispersed rapidly in all directions. Maximum distance of recovery from the point of release was 1.4 miles after 24 hours. After 10 days a few marked flies were observed at the release site.

Face flies were first observed in the field in May but populations remained low until late July, whereas in 1963 high populations developed by mid-June. Populations on cattle remained fairly constant in August but fluctuated with changes in temperature in September. When temperatures were below 70° F very few flies were active. Maximum populations of 13 per cow were noted on September 29, a warm day, but on this date flies began to hibernate in one location (grain storage building) although the inside temperature was 90° F. However, the flies moved in and out of the building and small populations persisted on cattle until the advent of cool weather in late October.

Studies were continued in 1964 on the insect fauna in cattle droppings on 3 of 8 farms studied in 1963. Over half of the insects collected in droppings were Diptera, 42% of which were face flies. The total insect population in 1964 was substantially higher than in 1963 but the number of face flies in droppings was almost identical. Parasitism of face fly pupae was low (0.7%) in 1964 but adult populations on cattle were constantly lower than in 1963. Reasons for this were not determined. In addition to face flies, droppings contained substantial numbers of aphodius beetles and Sarcophaga larvae. About 16% of Sarcophaga pupae were parasitized by Hymenoptera and Staphylinidae. Collections showed 10 species of Diptera, about 30 species of Coleoptera and 3 species of parasitic Hymenoptera.

In Maryland, outdoor behavior of face flies was studied, both on herds in the field and on a single animal confined in a cage with a known fly population. The data indicates the following: Only a small proportion, usually less than 10-15% of the total face fly population actually annoys cattle at any given time. Female flies visit the animals much more frequently than males, but males do cause some annoyance. Three- and five-day-old females visit the cow more frequently than 1-day-old females. The number of flies present on cattle is most closely related to the activity of the animal, with the greatest annoyance when the cattle are resting quietly. Although flies visit the face most frequently, they do rest on other parts of the body in considerable numbers. An evident peak of activity of released colonized flies occurred early in the morning, but that of wild flies appeared more evenly throughout the day. All flies leave the cattle at dusk while natural light levels are still quite high.

Additional studies of the nocturnal habits of face flies confirmed that they rest on the foliage of trees at night. Although the flies are readily attracted (about 80%) to blacklight in confined spaces, those found resting on foliage at night do not respond in this manner. Electrocutor grid traps with black light lamps placed in trees attracted less than 1% of the released population in 48 hours. Investigations of the factors affecting this change in bahavior may provide information useful in control.

Laboratory tests of the mating activity of female face flies indicate that sterilized males compete effectively with normal males in mating. Also, females appear to mate only once if they are inseminated during their first mating. Examination of females observed attempting to remate showed that only 5 - 10% of all females had not received any sperm during their first mating. This characteristic indicates that use of sterilized males should be effective in preventing reproduction. A laboratory trial using a ratio of 8 sterilized males to 1 untreated male to 1 untreated female resulted in a 94% reduction in pupae.

When marked face flies were released near cows in Maryland about 1 hour before sunset, a few marked flies and a few wild flies were noted on the cows for several minutes after release. They left the cattle when the level of natural light was relatively high, indicating that artificial light would probably not be an effective attractant. In dispersal studies, 24,000 individually marked face flies were released in 4 different areas. Several marked flies were found 2 miles from their release point after 24 hours. One fly was found 4 miles away after 5 days. Searchers for marked flies during the late fall showed that they spend the night in trees and tall weeds, rather then in or around barns.

5. Horn Fly. In Texas, a number of adult horn fly diets were compared for suitability in maintaining laboratory colonies. Diets composed of bovine blood, a saline extract of ground beef muscle and antibiotics or bovine blood, ACD, and cholesterol proved most satisfactory. Flies consumed more, reproduced better and survived longer on these than any other diets tested, including the standard (citrated blood, tissue fluid and antibiotics). Liquid extracts from manure of cattle fed fresh oat and dry alfalfa, sorghum and prairie hay were poured over cotton gauze pads and compared for suitability as horn fly larval media. The oat and alfalfa extracts produced pupae that averaged 3.16 mg in weight as compared to 2.5 mg and 2.1 mg for those reared on sorghum and prairie hay extracts, respectively. In additional tests in which the pH of the media was adjusted, horn fly larvae survived and developed equally well when pH's ranged from 6.0 to 9.0 but none developed in pH's 5.5 or less or 9.5 and above.

In Texas, studies showed considerable variations in the color of eggs deposited in manure by horn flies. Counts indicated about 84 percent of the eggs were dark brown, 13 percent tan and 3 percent yellow and white. The eggs were equally viable and equally capable of producing progeny.

In Texas, pressuring of successive generations resulted in a gradual increase in adult tolerance to ronnel. By the 20th generation a dosage of 0.1 µg/fly caused only 32-56 percent mortality. By the 28th generation tests showed an LD 50 of 0.3 µg/fly or about 50 times that of a normal susceptible strain.

6. Screw-worm. Research was continued on the screw-worm fly at Mission, Tex., in support of Southwestern screw-worm eradication program. Special studies were continued to develop a strain of flies resistant to starvation. Continuous selections resulted in a gradual increase in resistance to starvation until only negligible mortalities occurred in 72 hours in the 19th generation, in 96 hours in the 36th generation and in 144 hours in the 40th generation. Substrains removed from selection in the 18th and 30th generation retained their ability to withstand starvation. The sexual vigor of starvation resistant flies decreased gradually as resistance increased but the substrain removed from selection showed almost normal vigor 7 and 9 generations later. When females of the 27th generation of the resistant strain were held with males of the same strain under 96 hours of starvation no viable eggs were produced. When females were fed the hatch of eggs was 38% as compared to only 14% when only males were fed. When both sexes were fed hatch was 57% or about the same as that for fed unselected females and males.

Studies were continued on the mating behavior. It has been generally believed that female screw-worm flies mate only once, however, close observations showed that a high percentage of the female flies that mated with 1-day old males (inmature) mated a second time with mature males. On the other hand only 2 of 110 females first mated to mature males remated, 1 willingly and the other under duress. Egg viability was only 24% when females were mated with 1-day old males but increased to 65% when females were remated with mature males. When 1-day old males were exposed for 8 hours with 3-day old (mature) females and then replaced with mature males egg hatch was only 52%, compared to 89% for matings of mature males and females. These results indicate that females seldom remate if their first mating is satisfactory, i.e. with a mature male.

Competitiveness of irradiated (R) and non-irradiated (N) males was compared in multiple mating of females. Frequency of remating was increased by subjecting females to starvation periods of 20 to 24 hours and all matings were confirmed by observation. Females mated first with R males and then with N males averaged 33% to 48% fertility in 2 tests, compared with 75% to 85% for the controls. However, in the reciprocal matings fertility was 80% in both tests, indicating that R males were not competitive with N males. Mortality and fecundity of starved controls subjected to single matings were normal; however, 33% to 71% of the remated females failed to survive. Actual cause of death following forced second matings has not been determined but these observations help to clarify SAG test results in which aggressive males greatly accelerate female mortality.

Occasionally mating and fecundity studies have been conducted in which oviposition was induced immediately after copulation. Since duration of sperm storage in some mammalian females greatly affects fertility, this possibility was investigated in screw-worms. In 2 out of 3 tests fertility ranged from 47% to 55% when oviposition occurred within 3 hours of copulation, compared with 78% to 86% at 24 hours. In a third test fertility was 59% within 4 minutes but ranged from 80% to 97% from 3 hours to 4 days. Although the influence of sperm storage on fertility has not been clearly defined, a minimum 24-hour period between copulation and oviposition has been adopted in mating and fecundity studies.

Three screw-worm males selected at random from the Florida colony mated a total of 72 females each. All matings were confirmed by observation during daily 4-hour sessions. Two males ceased mating at 7 days when excessive wing damage appeared to interfere with proper positioning, and the other was dead on the 7th day. Peak mating activity occurred during the 3rd to 5th days. Total matings ranged from 1 on the 1st day to a maximum of 20 on the 3rd day; however, from the 3rd to 6th days fertility primarily occurred only among the first 7 matings. Total females fertilized per male (hatch 1% to 98%) ranged from 18 to 24. These results are in agreement with previous studies in which mating activity was evaluated only on the basis of hatching records. Although female remating seldom occurs following copulation with a mature, spermatous male (unless the females are too weak to elude the male), almost 70% of the females in the above test remated.

Studies were conducted to compare the ovarian growth of normal and starvation-resistant female flies under starvation, feeding after starvation and continuous feeding. Under starvation very little growth occurred but when food was provided the starvation-resistant females showed faster development than those of the normal strain. Ovarian growth was comparable when the two strains were fed continuously. Ovarian growth was more rapid in females fed meat than in those on a meatless diet.

Bioclimatic studies showed that both normal and selected strains of newly emerged screw-worm flies survived well when held 30 to 45 hours at 32° F, mortalities being 25 and 10 percent, respectively. Oviposition and egg viability of surviving females was not affected by the exposure. Exposures of 48 to 72 hours at 105° F caused 46 and 38% mortalities of the two strains and oviposition by the survivors and viability of eggs was greatly reduced.

Laboratory studies showed that male screw-worm flies reared on horse meat were about 25 percent larger (based on weight) than flies reared on the liquid medium now used for mass production of screw-worms for use in Southwestern control program. Also the meat-reared males were able to mate with 40% more females than the liquid-medium reared flies, although no difference was observed in the number of mating attempts by the two strains.

A preliminary investigation made in January in Mexico indicated that in average winters there is very little screw-worm overwintering in the north-

western corner of Sonora or in the northern part of Lower California. However, in the coastal regions of Sonora between parallels 28° and 30°30', it is seldom cold enough to completely eliminate screw-worms. Instead they are confined to certain bowl-shaped terrain features known locally as bahias. These bahias are warm and moist and furnish preferred pasture for cattle both winter and summer. It appears that the bahias have somewhat the same relation to screw-worm survival as the river valleys in southwest Texas, but it is not known to what extent screw-worms move from one bahia to another at different times of the year.

In Texas field tests were conducted to study the relative dispersal abilities of irradiated normal and selected (starvation-resistant) strains of screw-worm flies. A total of 330,000 flies of each strain were distributed by airplane in two releases along a 6-mile swath on a large improved range area. Totals of 1659 selected strain flies and 2252 normal flies were recovered from traps, indicating that the normal flies weremore vigorous than those of the selected strain.

In August 1964, a test was initiated in a 2000-square mile area in Veracruz, Mexico, to determine the efficiency of dispersal of flies dropped from aircraft at 8-mile swath intervals as compared to the standard intervals of 2-4 miles. Eleven releases of 400 males per square mile were made between August 29 and November 6. Since the area was naturally infested with screw-worms, efficiency was based on the percentage of sterile egg masses collected on wounded animals in pens located 0.1, 2 and 4 miles from release lines as compared to that on animals in a control area. Fly traps were operated at each pen from October 19 until December 1 to obtain data on the relative abundance of wild and released flies. The number of egg masses was fairly high early in August but declined rapidly with the onset of hot-dry weather and remained fairly low until late September. The numbers of egg masses began increasing with favorable weather early in October and remained fairly high until termination of the test. A few sterile egg masses were collected after the first male release. The percent sterility increased steadily thereafter to a peak of 68 percent by October 2, and declined gradually to about 20 percent at time of the last fly drop on October 27. There were no significant differences in egg mass sterility at different distances from lines of dispersal, indicating that the flies dispersed uniformly and apparently rapidly. All trap catches showed larger numbers of sterile flies than wild flies from October 26 through November 6 but native flies outnumbered sterile flies in all traps by November 14. Only 2 sterile flies were present in collections on November 19 indicating a maximum longevity of about 3 weeks.

7. Cattle Grubs. In Texas studies were continued to develop laboratory techniques for the rearing of cattle grub larvae. Several media consisting of agamma calf serum combined with various chlorides, glucose and other materials proved highly satisfactory for in vitro rearing of cattle grub larvae. Some first instar larvae survived as long as 120 days in these

media and many doubled or tripled their weight during this period. Small numbers molted to the second instar and survived another 60 days.

A major problem in in vitro rearing of cattle grub larvae is the development of bacterial infections which kill the larvae and considerable effort was devoted to the development of ways and means to solve this problem. Ultraviolet light effectively controlled bacteria but caused high mortalities of grub larvae. Various antibiotics and merthiolate were partially effective but reduced longevity of larvae. Studies are continuing.

Studies were undertaken to determine the absorption and ingestion of food by 1st instar cattle grub larvae confined in artificial media containing P_{32}-labeled phosphate. Examinations showed that the larvae contained much higher concentrations of radioactivity in the blood and integument than in the gut at any given time interval, indicating that most if not all intake was by absorption. Similar results were obtained with media containing dyes or carbon black. Dissections of larvae and examinations of the foreguts and hindguts failed to reveal any evidence of a lumen.

In Texas all grubs from the backs of a number of cattle imported from Wyoming were extracted and indentified. The population consisted of 83% H. lineatum and 17% H. bovis.

8. Horse Flies and Deer Flies. In Mississippi, studies were conducted to develop a suitable medium for the rearing of tabanid larvae in the laboratory. Sand and soil was unsatisfactory because larvae did not survive and develop well and they could not be observed without removing them. A semi-solid agar medium proved fairly satisfactory but it tended to harden with age. Of other media tried the most satisfactory consisted of small glass beads covered with water. The larvae survived well, were able to move freely, the container and larvae were easily cleaned, and the larvae were easily observed while moving and feeding on fly larvae or worms. From 60 to 85 percent of larvae of T. americanus, T. abdominalis, T. proximus, and C. crepuscularis survived by this rearing technique. Survival of 4 other species was much lower. Larvae of T. vittiger schwardti showed the most rapid development from egg to mature larvae. Length of larval instars ranged from 5-6 days for the 1st, 2nd, and 3rd to 52 days for the 7th. The average time from egg to mature larvae was approximately 120 days. Attempts to mate adults were unsuccessful.

In additional tests larvae of T. vittiger schwardti developed from the 1st through the 4th stage fairly rapidly in from 5.0 to 11.3 days per stage but development of succeeding stages required successively longer times, ranging from 18.3 days for the 5th to 60.5 days for the 8th stage. Length of the pupal stage ranged from 5 to 10 days, averaging 7 days. Total days from egg to adult ranged from 143 to 209 days, averaging 189 days.

In Mississippi, after many unsuccessful attempts a method was developed for obtaining eggs from engorged adult tabanids. Engorged adults are placed in

a screen cylinder which is then inserted into a wide-mouth gallon jar containing 2-3 inches of water-saturated sand covered with a screen shield. The upper end of the screen cylinder projected 4-5 inches above the mouth of the jar and is covered with cheesecloth. This arrangement provides the range in humidity necessary to satisfy the different water requirements of various species. With this arrangement eggs were obtained from 8 species and records made of the numbers of egg masses laid and days to hatch. The number of egg masses ranged from 1 for T. atratus and 4 other species of 10 for T. abdominalis. Eggs of T. lineola and T. vittiger schwardti hatched in 2-3 days but those of other species required from 5 to 7 days. Newly hatched larvae of most species survived and developed well in thin layers of agar in plastic dishes.

In Mississippi, adult horse fly populations increased gradually during the spring, reached a peak during late May and early June, and declined rapidly during late June and early July. Seven species were present during this period but the predominant species was T. vittiger schwardti followed by T. lineola, T. fuscicostatus, T. equalis, T. abdominalis, T. americanus and C .crepuscularis.

B. Insecticidal and Sanitation Control

1. Mosquitoes. In Oregon 56 compounds were screened on cattle by the "spot test" method for effectiveness as toxicants and repellents against adult mosquitoes. None of the materials were outstanding toxicants. Four materials--ENT 27194, ENT 27195, ENT 27196 and ENT 28086--showed fair to good repellency at dosages of 500-1000 mg/ft^2.

At Gainesville, Fla., the developmental program on insecticidal compounds for mosquitoes was continued. A large number of new candidate materials were tested in the laboratory for their potential as larvicides and adulticides. Many proved promising for further development.

Tests were conducted to evaluate fogs of naled, fenthion, Bayer 39007, and malathion against caged salt-marsh mosquito adults. Bayer 39007 was the most effective in these tests followed by fenthion, naled, and malathion. A field test in which different formulations of malathion were applied by airplane at a ratio of 0.05 lb/acre showed a reduction of 81% in population levels with the fog oil formulation, 76% with fuel-oil formulation, and 44% with water emulsions. Airplane sprays tests with four organophosphorus insecticides on adult salt-marsh mosquitoes indicated all were highly effective at low dosage rates. Comparison of the effectiveness of aerial sprays of malathion applied as a thermal fog and fuel oil spray showed the latter to be more effective.

Tests were conducted in the rice-growing area near Stuttgart, Ark., to evaluate the residaul effectiveness of some new insecticides against natural infestations of Anopheles quadrimaculatus. The insecticides were applied to the walls and ceilings of farm buildings at 200 mg/ft^2 as wettable powders and/or emulsions. Pre- and post-treatment counts were made of the mosquitoes

resting in the treated buildings as well as in six untreated buildings which were utilized as checks. An emulsion of Hercules 9485 was highly effective, causing 99.7%-100% reductions for at least seven weeks. As this compound was not available at the beginning of the series, it was applied from two to three weeks after the other treatments. A wettable powder formulation of Shell SD-8530 caused reductions of 98-100% for 9 to 10 weeks. A malathion wettable powder used as a standard caused 100% reductions for 4 weeks and 96-100% reductions (avg. 99.0%) throughout the 9-week test period. Hercules 9326 emulsion caused 100% reduction of the mosquito infestations for at least 9-10 weeks in two buildings but in a storage shed produced only 91% to 96% control the 6th and 8th weeks. Wettable powder formulations of CELA S-1942 and CELA S-2225 were slightly less effective, with control falling below 70% in some buildings by the sixth week. Shell SD-8211 was highly effective in two buildings but not in a third.

Tests were also conducted to evaluate the residual effectiveness of treated cheese-cloth when applied to the walls and ceiling of buildings in the same area. The cheese-cloth, which was purchased in rolls 3 feet wide, was first flameproofed and then impregnated with Bayer 39007. Buildings in which a complete coverage of treated cheesecloth had been used showed 100% reduction of the mosquito populations for the full 10-week duration of the test. All buildings treated by means of a strip of cloth around the edge of the ceiling and in the corners showed 100% reduction for 5 weeks, and 82 to 99% control for the next 5 weeks. Buildings treated by means of cloth around the edge of the ceiling only, or in the corners only, showed 98 to 100% reduction of mosquitoes for 5 weeks, and 84 to 100% control for the next 5 weeks.

At Corvallis, Oreg., tests were continued on the development of more effective insecticides for mosquito control. In field tests against snow-water Aedes mosquito larvae, excellent results were obtained with lindane, BHC, and fenthion at 0.05-0.1 lbs/acre. Abate and Dursban were generally less effective. Against mosquito breeding in log ponds, granular formulations of fenthion and abate gave excellent control. Both were also effective when applied with a pump oil can. In cooperative tests in California low volume airplane sprays of malathion and fenthion showed considerable promise as mosquito larvicides.

Infusions and hot water extracts made from several tree species were tested for toxicity against Culex tarsalis larvae. Toxic elements were found in Western red cedar, ponderosa pine, and to a less extent in lodgepole pine and redwood. Similar hot water extracts made from Douglas fir, Sitka spruce, Western hemlock, big leaf maple, red alder, and white fir were nontoxic to larvae. Studies are in progress to characterize the toxic principles through fractionation of extracts.

None of 7 analogs of DDT showed promise against resistant Culex tarsalis larvae.

At Corvallis, Oreg., experiments with C^{14}-TDE indicated that both susceptible and DDT-resistant Culex tarsalis larvae detoxified TDE by dehydrochlorinative and oxidative routes. The results suggested that resistance to DDT and related compounds in tarsalis involves a mechanism other than dehydrochlorination.

Studies were continued in the search for compounds that would act as synergists to overcome insecticide resistance in mosquitoes. Of a number of phosphorus esters, butyl-containing esters were most effective although other types showed activity.

2. House Fly. At Gainesville, Fla., research was conducted on the development of safer, more effective insecticides. Materials were evaluated in the laboratory as contact sprays and residual toxicants as a basis for selecting promising insecticides for field evaluations. Evaluations as residual toxicants included different formulations of the materials. Twenty-one promising compounds were tested as house fly larvicides in manure under caged poultry. Four compounds were highly effective as larvicides.

Residual tests were conducted with emulsions of malathion, diazinon, ronnel, dimethoate, naled, fenthion, and Bayer 41831, and with wettable powders of malathion and Mobil MC-A-600 against house flies in barns. All were applied at 100 mg/ft^2. Control was considered satisfactory as long as the reduction produced by the chemical was 75% or above. Dimethoate residues gave satisfactory control on most occasions for 14 days, after which they were ineffective. Mobil MC-A-600 wettable powder gave satisfactory control for 14 days in one test, but failed as early as the 1st day in a replication of the test. Other compounds gave satisfactory control for shorter periods.

At Corvallis, Oreg., research was continued to find compounds effective in synergizing organophosphorus insecticides and resistant strains of house flies. A number of different types of phosphorus esters were effective when combined with either malathion or parathion in overcoming resistance in house flies to these two compounds. Materials synergizing malathion against resistant insects differed considerably from those known to potentiate the toxicity of malathion to mice or cause ataxia in poultry.

3. Stable Fly. In Texas 119 new compounds were screened in spot tests on cattle for repellency and toxicity against the stable fly. Nine of these compounds were Class IV toxicants at concentrations of 0.5 percent or lower. Materials effective at a low concentration of 0.1 percent were Shell SD-8967 and SD-9102, and Cela S-1942. The only effective repellents at a 5% concentration were ENT-nos. 28086, 28087 and 28093. Special tung oil formulations from a commercial source proved both non-repellent and non-toxic to stable flies. Spot tests were conducted to evaluate a number of materials as extenders for pyrethrum and conventional insecticide. One material, Armour ARD-226, increased the repellency of malathion and extended residual effectiveness slightly. Other materials were ineffective.

In Texas, large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low valume sprays for the control of stable flies. Treated cattle were exposed for 24 hours periodically to flies in large cages but otherwise were kept outdoors. Pour-ons (8 oz/animal) of coumaphos were effective against stable flies for 15 days. Pour-ons of fenthion and ronnel were only slightly less effective, but carbaryl was effective for only 3 days. Conventional 2-quart spray applications of 0.1 percent Stauffer R-5723 and 1 percent Mobil MC-A-600 and Cela S-1942 were effective 3 to 10 days against stable flies. In similar cage tests with low volume sprays (23-69 ml/cow) 1 percent applications of 0.5 percent methoxychlor, malathion and DDT controlled stable flies for 3-6 days as compared to 1-3 days for 7 other materials.

Comparative tests were run with the WHO test kit to compare the susceptibility of stable flies to 12 insecticides. Five of the materials were equal in toxicity to ronnel (standard) and 3 were more toxic, namely, Shell SD-8436, SD-8447 and SD-8448.

At Gainesville, Fla., chemicals were evaluated in the laboratory as potential larvicides for the control of stable flies. Approximately 150 compounds were tested by exposing larvae to these compounds when they were incorporated into the larval rearing medium. Approximately 20 of the compounds were highly effective, approximately equal in activity to a standard, Bayer 39007. Tests with calcium arsenate as a larvicide indicated that it did not compare favorably with other compounds that were evaluated. In addition approximately 120 compounds were evaluated in laboratory tests as adulticides and some 20 were highly effective. Several of the more promising adulticides were tested as fogs against caged adults under field conditions, indicating the potential of these materials for controlling natural populations. Comparative tests of adulticides against caged insects indicated no differences in effectiveness of thermal vs. non-thermal fog applicators or between fuel-oil and water-based formulations. A contract was negotiated with the Florida State Board of Health to conduct research on insecticides for controlling natural populations of stable flies under conditions found in the Gulf area of Northwestern Florida. The research contract will take advantage of research conducted at the Gainesville Laboratory and evaluate insecticides under practical field conditions.

4. Face Fly. In Nebraska bioassay tests showed that the addition of 1.0 ppm of Thiabendazole, a new parasiticide for livestock, to manure prevented the development of face fly larvae to the adult stage. Concentrations of 0.1 ppm and lower were ineffective. The addition of 0.5% of Bacillus popilliae to manure had no effect on the development of face fly larvae but the addition of 1.0% reduced fly emergence by 24%.

5. Horn Fly. In Texas large cage tests were conducted to evaluate the effectiveness of various insecticides as pour-ons or low volume sprays for the control of horn flies. Treated cattle were exposed periodically for 24 hours to flies in large cages but otherwise were kept outdoors.

Pour-ons (8 oz/animal) of coumaphos were effective against horn flies for 20 days. Pour-ons of fenthion and ronnel were slightly less effective and carbaryl was effective for only 3 days. Conventional 2-quart spray applications of 0.1 percent Stauffer R-5723 and 1 percent Mobil MC-A-600 and Cela S-1942 were effective for 7-10 days. In comparative tests low volume sprays of 5 and 10 percent ronnel gave 100 percent kill of horn flies in 24 hours whereas 1 percent sprays gave only 86 percent kill.

In Oregon large cage tests were conducted to determine the effectiveness of certain tacky polybutanes against horn flies. All of the materials were repellent the first day after application but only one--Amoco H-120--showed repellency for 2-3 days. Additional cage tests were run to evaluate the effectiveness of 1/2 inch, 1 inch and 1 1/2 inch plastic collars containing 20 percent dichlorvos for the control of horn flies. The collars reduced horn fly populations by 86 to 100% in 3 hours and 100% in 24 hours and were still completely effective after 2 weeks of wear and exposure.

In the mid coastal areas of Texas pour-on applications of 2 ounces per cow of 8% Ruelene and 5% ronnel gave excellent control of horn flies for 6 days, while applications of 4 ounces were effective for 11 days. In central Texas conventional sprays of 0.3 percent Ciodrin, 1 percent trichlorfon, 1 percent Cela S-1942, 0.5 percent carbaryl and a pour-on of 1.0 percent coumaphos provided effective control for 2 weeks. Several other materials were effective for about 1 week. Similar treatments gave slightly shorter periods of control in humid coastal areas.

Low volume sprays of 5 and 10 percent ronnel applied to 1 square foot areas (withers and brisket) gave good control of horn flies but lower concentrations were unsatisfactory. In other tests excellent control of horn flies was obtained by treating only part of the animals in herds with 8% Ruelene at 1 oz/100 lbs body weight as a pour-on treatment. In one test the treatment of only 2 cattle in a herd of 50 reduced the overall horn fly population by 75 percent in 10 days.

In Texas bioassays were run to determine the toxicity to horn fly larvae of the manure from cattle that had been fed seven insecticides at varying rates for 10 days. All of the materials reduced larval survival but only Bayer 37341 and Stauffer R-3828 at 5 mg/kg daily gave 100 percent mortality.

In Mississippi, conventional spray applications of 2 quarts per cow of 0.375 percent coumaphos, 0.2 percent Bayer 9017, 0.5 percent Hooker 1422 and 0.5 percent methoxychlor provided effective control of horn flies for 10 days. The lowest test concentrations of 0.06 percent coumaphos, 0.06 percent trichlorfon, 0.05 percent Bayer 9017 and 0.1 percent fenthion were effective for 6 to 8 days or about as long as 2- to 4-times higher concentrations. In comparative tests back rubbers treated with 0.25, 0.5 and 1.0 percent Bayer 9017, 0.5 and 1.0 percent coumaphos and 0.5 percent ronnel maintained effective control of horn flies for 7 to 8 weeks, whereas these treated with 0.5 percent dimethoate and 0.5 percent Famophos were effective only 4

weeks. Observations suggested that loss of effectiveness was due to loss of the oil solvent since the odor of insecticide was still detectible. Retreatment of each backrubber with 1 gallon of oil resulted in 4 weeks additional control, thus confirming the above observation.

In Mississippi, a series of tests were run to compare the effectiveness of 0.5 percent oil solutions of 10 insecticides applied in low volumes by automatic sprayers. Single applications of ronnel, Ciodrin, Dioxathion, Bayer 9017, Shell compound 4072 and Dowco 175 provided excellent control of horn flies for 6.5 to 8.5 days. Other materials were equal or slightly less effective than toxaphene (standard) which gave satisfactory control for 5 days.

6. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Of twenty new compounds screened for larvicidal effectiveness at 10, 1.0 and 0.1 ppm in screw-worm larval medium, four were highly effective, killing all the larvae at 1.0 ppm, namely, Shell SD-8964, Shell SD-8988, Shell SD-8967, and Geigy GS-12968. None of the compounds screened were effective at 0.1 ppm.

In field tests in Mexico, cattle infested with 1- and 2-day-old screw-worm larvae were sprayed or dipped in promising insecticides. Shell Compound 4072 in a dip or spray at 0.1% killed all the larvae, as did Cela S-1942 in a 1.0% spray. Hooker HRS-1422 as a 0.25% spray and Shell Compound 4072 as a 0.08% in a dip were fairly effective but permitted a few larvae to survive. Telodrin as a 0.05% spray killed both 1- and 2-day-old screw-worm larvae but it also killed 3 of the 4 cattle treated. Bayer 37289 (0.25% spray) and Bayer 38333 (0.1% spray) killed all the larvae in one test, but not in another. Sprays containing 0.1% of ethion, 0.1% of Dowco 175, 0.05% of dimetilan, 0.01% of Bayer 29952, 0.01% of Stauffer N-2790, or 0.01% of Bayer 38156 were ineffective.

Previous research has shown that sprays containing 0.1% or higher concentrations and a dip containing 0.1% of Shell Compound 4072 are effective screw-worm larvicides. In new tests in Mexico, cattle with wounds containing 1- and 2-day-old screw-worm larvae were dipped in vats containing either 0.05% or 0.1% Shell Compound 4072. At examination 24 hours after treatment, no live larvae were found in wounds on cattle dipped in 0.1%. All 1-day-old larvae were killed by 0.05%, and live 2-day-old larvae were found in only 1 of 16 wounds.

7. Cattle Grubs and Other Bots. Research was continued in Texas and Oregon to develop more effective insecticides for the control of cattle grubs and other bots affecting livestock. In Texas 113 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larvae of Cochliomyia macellaria and Phormia regina. Ten materials showed systemic activity in one or both types of administration. The most effective materials, dosages and routes of administration were as follows: Shell SD-9129, 5 mg/kg,

0 and SC; Spencer S-6900, 25 mg/kg, 0 and SC; and Cela S-2225, 25 mg/kg, 0. Seven other materials were effective at dosages of 50 to 200 mg/kg.

In Texas field tests were conducted on small numbers of Government-owned cattle (2 to 4) to evaluate the effectiveness of a number of materials that had shown promise in screening tests and of several older effective materials administered in different ways at several dosages. Materials giving 91-100% control of grubs when administered in the feed for 10 days were as follows: Bayer 37341 and Bayer 37342, 1.0 mg/kg; and Famophos, menazon, and Vamindoate, 5.0 mg/kg. As drenches, Cela S-1942 at 100 mg/kg and Shell SD-8949 at 50 mg/kg gave 91 and 100% control, respectively. Menazon as a 1.0% spray gave 100 percent control. Other materials were ineffective.

In Texas field tests were conducted on cooperative cattle on 9 ranches with several experimental materials and with a number of older systemics administered at different dosages in several types of formulations. In pour-on tests materials, concentrations, formulations, and the lowest rates of application that gave 95-100% control were as follows: 4.0% oil suspension of coumaphos, 10 mg/kg; 15.5% water emulsion of Bayer 37342, 100 mg/kg; 4.0% water emulsion and oil suspension of Ruelene, 25 mg/kg; 7.75% water solution of trichlorfon, 50 mg/kg; and 10.2% oil suspension of ronnel, 150 mg/kg. Conventional spray treatments giving 92-100% control of grubs were 0.25% fenthion emulsion, 0.5% Rulene water suspension, 70 mg/kg, 0.25% Shell Compound 4072 water suspension, and 1.5% trichlorfon water solution, 250 mg/kg.

In Texas field tests were conducted to evaluate the effectiveness of 6 materials on Wyoming cattle infested with the northern cattle grub, Hypoderma bovis, as well as the common cattle grub, H. lineatum. Oral administration of Stauffer R-3828 at 25 mg/kg gave 90% control of grubs. The other materials were partially or completely ineffective.

In Oregon extensive field tests were conducted to evaluate the effectiveness of 7 insecticides as pour-ons and 2 as sprays for the control of cattle grubs. In these tests, pour-ons of 8% Ruelene in water emulsions at 52 mg/kg and in oil solutions at 25, 34, and 46 mg/kg per animal gave 99% control of grubs. Similar results were obtained with pour-ons of 8 and 12% trichlorfon at 30 and 45 mg/kg, and 2% fenthion at 7.5 mg/kg. Pour-ons of ENT 25482 at 9[illegible] mg/kg and Shell SD-8436 at 12 mg/kg gave 97% control of grubs and Shell SD-8447 at 121 mg/kg was only slightly less effective. Sprays of 0.1 and 0.25% Imidan® showed 88-89% control but lower concentrations were ineffective.

8. Horse Flies and Deer Flies. In Mississippi, sprays of 2 percent Ciodrin gave excellent immediate protection of cattle from horse flies but no effect was apparent after 3 hours. Applications of 1 percent Ciodrin plus 0.25 percent dichlorvos provided excellent protection for 7 hours and up to 24 hours in some tests.

9. Ticks. Studies were continued in Texas to develop effective systemics and conventional insecticides for use in the control of several species of ticks on cattle and other animals. A total of 114 new compounds were screened for systemic action by giving them orally (O) or subcutaneously (SC) at several dosages to guinea pigs infested with larval lone star ticks. Only 6 of the materials showed systemic effectiveness. The outstanding materials, dosage and method of administration were as follows: Velsicol FCS-13, 25 mg/kg, O and 10 mg/kg, SC; and Spencer S-6900, 25 mg/kg, O and SC. The other 4 materials were effective at dosages of 50-100 mg/kg by one or both methods of administration.

In Texas 160 insecticides screened in dipping tests against engorged Boophilus females to determine their effectiveness in preventing oviposition and/or hatch of eggs. A total of 44 of the insecticides were ineffective at the highest test concentration of 1.0%. The remainder were effective at 1.0% or lower concentrations. Materials that were effective at the lowest test concentration of 0.01% in preventing oviposition were as follows: Shell SD-8448 and SD-9102, and Niagara NIA-9227. Materials which permitted light oviposition but prevented hatching of eggs were as follows: carbophenothion, Stauffer R-2964, N-3727 and N-3794, Monsanto CP-40272 and Wm. Cooper 57-H-62.

Extensive field tests were conducted in Mexico to evaluate the effectiveness of 12 promising insecticides as sprays and/or dips for the control of Boophilus ticks on cattle. In dipping vat tests, concentrations of 0.05-0.1% of Shell Compound 4072 gave 100% mortality of flat and engorging stages and no live ticks were noted after 1 week, indicating that residual material killed all molting stages. Similar results were obtained with sprays of 0.1% Shell Compound 4072 and Dowco 175 and 0.01% Bayer 38333. Sprays of 0.25% Hooker HRS-1422, 0.1% ethion, 1.0% Cela S-1942, 0.25% Bayer 37289, and 0.01% Bayer 38156 were highly effective but a few ticks were still alive on treated animals after 1 week. Sprays of two materials--0.05% Teleodrin and 0.01% Bayer 29952--killed or severely poisoned cattle. Dimetilan and Stauffer N-2790 were not highly effective.

In field tests in Texas, sprays of 0.1% Shell Compound 4072 and fenthion, 0.25% Imidan and 0.5% Cela S-1942, malathion and toxaphene gave highly effective control of the winter tick, Dermacentor albipictus, on cattle. Little or no reinfestations developed on treated animals within 1 month after spraying.

In field tests in Texas, sprays of 0.25% coumaphos and Imidan, 0.03% diazinon, 0.1% Shell Compound 4072, 0.3% Ciodrin, 1.0% trichlorfon, and 0.5% toxaphene gave excellent immediate control of lone star ticks on cattle. Four other materials failed to give satisfactory control. Pour-on applications of 8.0% trichlorfon and 2.0% Hercules 7522, which are excellent systemic treatments against cattle grubs, were relatively ineffective systemically against ticks.

Extensive field tests were conducted in Texas to evaluate the effectiveness of 32 insecticides as sprays and/or dusts for the control of spinose ear tick, Otobius megnini, in the ears of cattle. All of the materials except Dri-Die and Dowco 175 dusts, and menazon sprays gave excellent to complete control of infestations of the spinose ear tick. However, the only treatments still showing effective control after 1 month were 5% dusts of coumaphos and Shell Compound 4072, 1% Hercules 7522 dust and 0.3% Ciodrin spray.

Small-scale field tests were conducted in Florida to evaluate several insecticides for effectiveness against the tropical horse tick (Dermacentor nitens), the vector of equine piroplasmosis. In these tests dermal and ear applications of 1% lindane, 0.3% Ciodrin, 0.25% Imidan and coumaphos, and 0.1% Shell Compound 4072 gave 100% immediate control of ticks but light reinfestations developed in all instances in 2 weeks. Dri-Die dust was ineffective. In systemic tests trichlorfon at 10 mg/kg a day for 10 days was completely effective in clearing ticks from the ears of horses. Fenthion at 5 mg/kg for 5 days, Famophos at 10 mg/kg for 4 days and Hercules 7522 at 5 mg/kg for 4 days reduced but did not completely eliminate tick infestations.

Surveys in southern Florida showed D. Nitens to be present at 4 of 15 locations examined but populations were high in only one. Insecticidal treatment of pastures and horses apparently have eradicated the tick in the other 11 locations.

10. Lice. In Mississippi 14 promising insecticides were evaluated by the spot test method against cattle lice. Three materials, Stauffer B-10046, R-5724, and R-5725 gave 100% immediate kills of motile lice but all permitted reinfestations to develop in 14 days. In field tests, two applications of 5% dusts of coumaphos, carbaryl and dioxathion 2 weeks apart eliminated lice on cattle. Similar applications of 5% methoxychlor gave excellent control but did not completely eliminate the lice.

In Nebraska, treatments of groups of cattle with 0.5% ronnel applied with a Bean Rotomist sprayer eliminated all motile stages of cattle lice. However, after 1 month light infestations were again present on some animals.

C. Insecticide Residue Determinations

1. Residue studies. In Texas tests were conducted to determine the levels of residues in tissues of cattle forced to use back rubbers treated with 1 and 2 percent ronnel-oil solutions four times daily for 28 days. Small average residues of 0.005 to 0.05 ppm were found in the fat after 2 weeks but only negligible amounts were present after 4 weeks and none whatever could be detected 2 weeks after treatments were discontinued. Residues in muscle, liver, kidney, heart, brain, and spleen were barely detectable after 2 weeks treatment and none whatever could be demonstrated thereafter.

Additional studies were conducted to determine the distribution of P^{32}-ronnel dermally on cattle forced to use backrubbers treated with this material. Comparisons were made between animals receiving 2 and 4 exposures per day for 4 weeks. Distribution as indicated by analyses of hair samples was very irregular but most of the insecticide was concentrated along and adjacent to the central back line and tip of the head. Cattle treated 4 times a day received about twice as much insecticide as those treated 2 times daily. No ronnel was present on the hair 2 weeks after the last treatments.

In Texas two tests were conducted to determine the sites of accumulation and amounts of residues in various tissues of cattle resulting from dermal sprays of 0.1 percent Shell Compound 4072 emulsion. In one test, the cattle were sprayed weekly for 12 weeks; in the other test they were sprayed 6 times at 2-week intervals. In the 12-weekly spray test, analyses 1 week after the first spray showed residues in the fat ranging from 0.007 to 0.045 ppm (average 0.02 ppm). Residues increased slightly with successive weekly sprayings to a peak average of 0.14 ppm (range of 0.097 to 0.196 ppm) after the eighth spraying. One week after the twentieth and last spraying residues in the fat averaged only 0.01 ppm (range 0.008 to 0.016 ppm). No residues were detectable 2 weeks after the last spraying. In the biweekly spray test analyses 2 weeks after the first and third sprays showed only 0.005 ppm in the fat but residues increased somewhat after the fifth and sixth sprays, averaging 0.117 and 0.133 ppm, respectively. Additional analyses of fat from animals slaughtered 2 weeks after the sixth and last spraying showed average residues of 0.065 and 0.112 ppm in renal and omental fat, respectively. No residues were detectable 4 weeks after the last spraying.

Analyses of tissues from a calf slaughtered 7 weeks after being sprayed with 0.25 percent Imidan showed no residues in samples of fat, muscle, heart, liver or spleen.

In Maryland, as a result of the detection of residues of heptachlor epoxide in the fat of cattle slaughtered at the Agricultural Research Center at Beltsville, analyses were made to determine if residues were present in the milk of three experimental dairy herds. The over-all average residue of heptachlor epoxide in the milk was below 0.01 ppm. Analyses of eight lots of hay and one lot of alfalfa pellets which were being fed to the cattle showed residues ranging from 0.00 to 0.048 ppm of heptachlor epoxide. Subsequent tests did not show detectable residues in the feed but low levels of heptachlor epoxide persisted in the milk of selected animals from experimental herds.

2. Toxicity Studies. Research was continued in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronix toxicity of insecticides and other chemicals to livestock.

Studies were conducted to determine the normal patterns of certain enzymes in average cattle as a prerequisite for studying the effects of chemical

poisoning on enzyme patterns and the effects of oxime-type cholinesterase reactivators (2-PAM, DAM and TMB-4). Cattle poisoned by Dioxathion caused elevations in activity of serum glutamic oxalate, pyruvate transminases, alkaline phosphatase, and blood beta lipoprotein but these increases were minimized by the administration of 2-PAM and TMB-4. All three test oximes prevented decreases in gamma globulin. These results indicated that TMB-4 was slightly more beneficial than 2-PAM. DAM did not appear beneficial at the levels (10-20 mg/kg) tested.

Cattle were poisoned with an oral dosage of dichlorvos to determine the effects on serum glutamic oxalacetic and pyruvic transaminase, aldolase and alkaline phosphatase. Oximes were given some of the cattle to determine their protection of those enzyme systemics. DAM and 2-PAM kept the enzyme activities of the mildly poisoned animals near normal, whereas TMB-4 appeared to cause an increase in activity above normal during the test. From the biochemical standpoint, it appeared that 2-PAM and DAM offered more protection to the enzyme activities than does TMB-4.

Cattle were poisoned with coumaphos and enzyme systems studied in serum. Some of the cattle received antidotal therapy with 2-PAM. Glutamic dehydrogenase, sorbital dehydrogenase, phosphohexose isomerase and serum arginase were studied in an effort to find significant enzyme activity alterations indicative of possible tissue change. No significant differences were noticed in the enzyme activities regardless of treatment. Mortality was reduced by 2-PAM in coumaphos poisoned animals, but this benefit could not be detected in the enzyme studies.

Additional studies were conducted to determine the effectiveness of these three oximes in reversing cholinesterase inhibition induced by organic phosphorus compounds. Each of the compounds was useful, but 2-PAM and TMB-4 appeared to be superior to DAM. Particularly encouraging was the beneficial effect of these compounds in cattle poisoned by coumaphos; usually such animals do not readily respond to atropine, the pharmacologic antidote. Oximes combined with atropine markedly increased the number and speed of recoveries.

Studies were conducted to determine the effects of the chemosterilant, apholate, when fed to Jersey cattle at 1 mg/kg daily throughout one gestation period. A deficiency of white blood cells appeared in one heifer after 80 daily doses and in the others after 110. One heifer died after 335 doses, one delivered a calf and died after 531 doses, one delivered prematurely and survived 581 doses, and the fourth delivered at term and survived 629 doses. In additional studies the new chemosterilant, hempa, appeared to be considerably less toxic than apholate, tepa and metepa, but it produced the same deficiency in white blood cells.

Studies were conducted to determine the toxicity of 72 insecticides to cattle, sheep and goats. Toxicities ranged from impossibly dangerous to

reasonably safe. Promising new insecticides showing low toxicities were Shell SD-8447 and Cela S-1942.

D. Biological Control

1. Mosquitoes. At Lake Charles, La., research on pathogens of mosquitoes has been conducted. Field collections throughout the area have shown infections of microsporidia in thirteen species including the genera, Culex, Aedes, Culiseta, Anopheles, and Orthopodomyia. Aedes grossbechi and Orthopodomyia signifera were new host records for a microsporidian. Spore sizes were determined and classification of the microsporidia studied. Transovarial transmission of microsporidian infections was studied in 12 species of mosquitoes and demonstrated in seven of the species.

The fungus Coelomomyces was found in field collections infecting larvae of Culex restuans, C. salinarius, Aedes vexans, A. solicitans and Culiseta inornata. Culex alinarius, C. restuans and Aedes sollicitans represent new host records for Coelomomyces. Field infection levels varied from very low to over 50 percent.

A polyhedral virus was reported from larvae of Aedes vexans and Psorophora ferox. Both species were previously unreported as hosts of viruses. Infection levels in the field were very low. A very lethal bacteria was collected from larvae of six mosquito species.

At Gainesville, Fla., two species of Thelohania were found in A. quadrimaculatus, one infecting the adipose tissue and the other the oenocytes. One species was found infecting the oenocytes of A. crucians larvae.

2. Face Fly. Studies in 1963-64 by personnel of the European Parasite Laboratory, Insect Identification and Parasite Introduction Research Branch, in France showed that the adults of Aleochara tristis, a staphylinid beetle, were predaceous on larvae of the face fly and other Diptera breeding in cattle droppings and that newly hatched larvae parasitize face fly pupae. Shipments of this species were received at Lincoln, Nebr., in January and February 1965 and immediate steps were taken to establish colonies. The adult beetles were caged under room conditions (80-85° F; 50-60% RH) and provided fresh cattle manure containing face fly eggs and small larvae. The beetles survived well, oviposited and newly hatched larvae were noted in 12 days. Face fly pupae were made available to the small larvae. Observations showed that the larvae quickly penetrated the face fly puparia and closed the entrance hole. The larvae consumed the face fly pupae and pupated within the puparia. Adult beetles emerged from the puparia through the area normally used by emerging flies. The combined larvae and pupal period was about 17 days and the adult preoviposition period of about 12 days. Large numbers of adults will be produced and released at selected field sites near Lincoln and studies made to determine the effectiveness of this parasite in reducing face fly populations.

3. Horse Flies and Deer Flies. In Mississippi, large numbers of tabanid larvae were collected periodically and transported to the Kerrville, Texas, laboratory to determine the number and species parasitized and the identity of the parasites. A total of 206 larvae, representing 4 species, were collected in October 1964. Only 3 larvae of T. atratus showed microsporidian infections. In subsequent collections in November 1964 and January 1965, microsporidian infections were found in some of 3 species of tabanid larvae, namely, T. sulcifrans, T. vittiger schwardti and T. atratus. The microsporidia in sulcifrans could not be identified but those infecting vittiger schwardti were of the genus Plistophora. Those infecting atratus were Thelohania sp. Healthy atratus larvae readily became infected when fed spores of Thelohania but not when fed those of Plistophora. Microsporidian infections could not be induced in fed or starved healthy larvae held at 10° C. At 25° C fed larvae readily developed infections but not starved larvae.

In Texas, approximately 300 tabanid larvae were collected and examined during the year and all were free of microsporidia and other parasitic infections.

Studies were conducted to determine the host range of microsporidia (Thelohania sp.) by feeding artifically infected C. macellaria larvae to different species of tabanid larvae. The normal host of this parasite, Tabanus atratus, readily became infected. Of 5 other species used, only one, T. americanus, developed typical spore infections. Similar studies were made with another microsporidian (Plistophora) but results were negative.

In Texas efforts to develop a reliable spore agglutination test based on sedimentation patterns formed by spores mixed and allowed to settle with rabbit antisera were unsuccessful. In other tests antimicrosporidian spore-rabbit serum reacted positively by agglutination against homologous antigen but further tests must be conducted to ascertain the specificity of the reaction.

4. Ticks. In Texas laboratory tests were conducted to determine the effectiveness of a dust preparation of the fungus Beauveria bassiana against 4 species of ticks. Adults and nymphs of Amblyomma americanum, adults of A. maculatum and adults of Dermacentor variabilis were highly susceptible to the fungus with 90 to 100% kills occurring in 7 to 10 days. Nymphs of the spinose ear tick Otobius megnini were not affected.

E. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., studies were continued on factors affecting the attraction of mosquitoes to their hosts and factors affecting the protection time from mosquito bites afforded by repellents. A large olfactometer was developed to study these factors as well as evaluate the efficacy of various attractant materials or factors.

The effort to develop effective space and systemic repellents was continued and slightly expanded. To date several materials show some space repellency to mosquitoes in that they prevent mosquitoes from penetrating 4-mesh screening. Materials exhibiting some systemic repellency were found and further tests will be made on these materials.

Studies were continued at Gainesville to evaluate materials as chemosterilants for mosquitoes and to evaluate the sterility principle of mosquito control. Tests with hempa indicated it would have little value as a residual sterilant for mosquitoes. Further selection and studies were conducted with the apholate-resistant colony of Aedes aegypti to clearly define the degree of resistance. This colony is at least 10 times as resistant to the sterilizing effects of apholate as the unselected, parent colony. Selections to increase resistance will be continued.

A sterile male release study of Anopheles quadrimaculatus was made in a semi-isolated area. This site was made more favorable by increasing the number of breeding sites and introducing additional wild stock of this mosquito to populate the area. When sterile males of wild stock were released in this area sterility of the natural population increased from a very low degree up to 42%. When the releases were changed to sterile colony males the sterility in the natural population decreased confirming earlier work showing behavior differences between colony and wild strains in seeking out wild females. Apparently a sufficient number of males was not released to reduce the population levels of A. quadrimaculatus.

Studies were continued on the evaluation of chemosterilants for mosquitoes at Corvallis, Oreg. The chemosterilant, hempa, was not highly effective in sterilizing Culex tarsalis larvae in that rates as high as 200 ppm were required. As a residual treatment in glass jars, 10 mg of hempa per square foot sterilized adult males completely, but adult females only partially. In wind tunnel tests against adults, a concentration of 10% caused high sterility, whereas a 5% spray caused only partial sterility and none was caused at 1% or lower. Males were generally more susceptible than the females.

At Corvallis studies were continued on sex and ovipositional attractants for mosquitoes. Preliminary tests indicated the presence of sex attractant in Culex quinquefasciatus, but further tests did not confirm its presence, nor the presence of a sex attractant in Culex tarsalis. Many mosquitoes are known to choose specific types of water for oviposition. Studies have shown that odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of Culex quinquefasciatus. These odors were not attractive to females of C. tarsalis. However, log pond water itself was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of C. quinquefasciatus but

not those of C. tarsalis. Gravid females of both species were more attracted to distilled water treated with 25 ppm of furfural than to water treated with 5 or 50 ppm.

2. House Flies. At Gainesville, Fla., research was continued on the development of chemosterilants and the sterility principle of control for house flies. Several hundrew new candidate compounds were evaluated in primary screening and secondary development tests and many were found to exhibit sterilizing efficacy against both males and females. Particular attention was paid to evaluating two compounds--hempa and hemel--as sterilants by several routes of administration. These two materials will sterilize both sexes of the house fly.

Two series of field tests were conducted at farms in Florida to evaluate the effectiveness of two chemosterilants, hempa and apholate, for the control of house flies. At the farm treated with hempa, house flies were reduced in abundance from 71 per grid to 0 within eight weeks. Grid counts remained at zero for the remainder of the test period. At the farm treated with apholate, population levels decreased from 200 per grid to less than 10 within 6 weeks and remained constant at a low level throughout the remainder of the test.

Ninety chemicals were screened as chemosterilants against adult house flies. Six compounds were toxic and 10 reduced the fertility to some extent in fly food or sugar. Thirty-two compounds, previously shown to sterilize house flies, were also tested again at higher or lower concentrations.

Tests were conducted with 21 compounds to determine their effectiveness as male house fly sterilants. Of the 14 chemicals Olin 53330, Squibb Olin 53331, Squibb Olin 53356, and Squibb Olin 53263 sterilized at a concentration of 0.05% in the sugar diet. Sankyo Co. RES-101 induced sterility at this dosage in sugar in the first two eggings.

Basic studies were continued on the cytological effects of chemosterilants on house fly reproductive systems and previous sectioning, fixing, and staining techniques have been used to study several new chemosterilants.

Olfactometers designed by Gouck and Schreck were used to initiate a search for more effective house fly attractants. Nineteen compounds were tested as house fly attractants, using Edamin as a standard. Beef protein concentrate was attractive to females but not to males. The other materials were not as effective as Edamin.

At Corvallis, Oreg., research was conducted on chemosterilants and attractants for the house fly and the little house fly. With the little house fly, hempa caused sterility as a residual deposit on glass at 50 mg/ft^2. However, the sterilizing dose caused some fly mortality. Higher doses were highly toxic to the adult flies; lower doses did not sterilize. When fed orally in the adult food to the little house fly, hempa was toxic at 0.25%

and lethal at 1.0%. High, but incomplete sterility was caused at concentrations as low as 0.01%. Topical treatments of hempa and hemel sterilized males of the little house fly without causing mortality, but not the females. In general treatments causing a high degree of sterility did not affect the mating competitiveness of females. Four known antioxidants exhibited little effect on egg production or egg hatch with the little house fly. Dosages of gamma radiation greater than 1000 r given to pupae of the little house fly prevented oviposition by emerging adults.

At Corvallis research was continued on the sex pheromone in house flies. The presence of a low titre of pheromone in extracts of pupae and young females was shown and confirmed. Higher activity of the pheromone was demonstrated in 3-day-old flies. The presence of the pheromone was shown in female flies from strains of different origin. Males of different strains reacted to extracts from females of different strains, though differences in behavior were apparent.

At Corvallis, Oreg., further studies showed that the sex pheromone in female house flies increased with the age of the flies, with the greatest increase occurring on about the third day after emergence. Comparative tests with benzene extracts of female house flies showed that high concentrations applied to pseudo flies inhibited male response. Similar inhibition occurred when normal extracts were applied to large surfaces within test chambers. Comparative tests with extracts of females with different solvents showed a much greater amount of the pheromone in hexane than in other solvent extracts. Efforts are being made to determine the chemical nature of the pheromone.

At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of house flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

3. Stable Fly. At Beltsville, Md., the effectiveness of electrocutor-grid screens placed in window plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of stable flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

4. Face Fly. At Beltsville, Md., additional tests of the responses of 3-day-old female face flies to monochromatic light confirmed that blacklight

is highly attractive under conditions of confinement and that wavelengths in the red and yellow spectral regions are unattractive. Refinements of the testing procedure and methods of data analysis are being made in an attempt to more clearly establish differences in attractiveness.

5. Horn Fly. In Texas, topical applications of 1 µg/fly of apholate sterilized adult horn flies. Lower dosages of 0.5, 0.2 and 0.1 µg/fly reduced the hatch of eggs but did not confer completely sterility. Males were more easily sterilized than females. In feeding tests, flies feeding over-night on a diet containing as little as 0.01 percent apholate were completely sterilized.

6. Screw-worm. In Texas 20 of 255 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worms when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration; some sterilized only one sex, and some sterilized both male and female flies. A review of past chemosterilant screening revealed that of the aziridine compounds screened, 92 were effective either by multiple-oral administration or topical application, or both. Only 8 were less than 100% effective when administered orally. In the future, routine use of topical applications in chemosterilant screening will be dropped in favor of multiple-oral administration.

It has been shown that there is a differential susceptibility between males and females sterilized with metepa. Tests with uredepa (ENT-50450) showed similar results, with the males about 9 times more susceptible than females on the basis of dosage/unit of body weight. There was also a greater variation in results obtained with females than with males. Starvation also increased the effectiveness of uredepa. The antifertility effects of another chemosterilant, ENT-25296, were enhanced by subjecting treated flies to periods of temperature stress (98° or 14° F), after administration of sterilizing or highly effective substerilizing dosages.

Male and female screw-worm flies can be sterilized by exposure to certain chemosterilants, but most of these chemicals adversely affect mating activity, longevity, or vigor. Three new chemosterilants were found that equal or surpass radiation in their effectiveness in achieving sterility of screw-worms. ENT-50838 applied topically provided a wide margin of safety between the minimum toxic quantity and the sterilizing dosage. Males sterilized with this material were hypercompetitive; they were sexually more aggressive than irradiated flies. The other two compounds, ENT-50716 and ENT-50781, were more toxic to the flies, but they were approximately equal to radiation as sterilizing agents.

In Texas 154 chemicals and other materials were screened as screw-worm attractants. Of these, 22 were equal to or better than the standard liver bait and require further evaluation. Some have been tested in the field in limited tests. The most outstanding were isovaleraldehyde, ethyl

isovalerate, and an ethanol extract of the flowers of Yucca treculeana. Liver-baited traps were seldom as effective as traps containing these materials. The presence of blooming wild flowers interfered with these tests; tests made during peak blooming seasons usually gave negative results. results.

The presence of a pheromone produced by males that is attractive to virgin female screw-worm flies was confirmed. In Texas, by means of the cold-trap method, 3500 ml of condensate were collected over a 5 1/2-month period from a cage containing virgin male screw-worm flies. Benzene and chloroform extracts of the condensate were capable of changing the behavioral pattern of virgin female flies. Sexually mature females in the presence of the odor go through "searching" motions and finally behave as in an aggressive mating "strike". Young (1-2 days old) females gave little or no response to the male odor, but 3-day-old females exhibited a definite activity, including the imitation of "male-type strike". Four-day-old females reverted to the response of 1-day-old females, but activity increased again in 5-day-old females. The greatest response was observed in 6-day-old females, with 7- and 8-day-old females showing a decrease in total number of "strikes". This decrease may have been due to wing damage, normal at this age. Other studies are in progress, including fractionation of the extracts to find the effective material. Another extract, made by filtering the air in the eradication colony room, brought a response on the part of both males and females.

7. Ticks. In Texas studies on the effects of radiation on lone star ticks showed that dosages of 250 and 500 r did not affect fertility of adults treated 1 week after molting from nymphs. However, a dose of 500 r sterilized females 1 day after engorging. Doses of 250 and 500 r had no effect on engorging and molting of nymphs or on fertility of resulting adults. However, at 1000 r the percentage molting of nymphs was reduced and resulting adults did not engorge or lay viable eggs.

F. Insect Vectors of Diseases

1. Anaplasmosis. Studies were continued in Texas and Mississippi in cooperation with the Animal Disease and Parasite Research Division and veterinarians of the State Experiment Stations to correlate the presence and abundance of insects and ticks with the incidence of anaplasmosis in herds of cattle. In Texas, monthly surveys were made to determine the identity and abundance of external parasites on infected and clean (segregated) herds of cattle. Lone star tick populations were light on cattle in January, increased gradually during February, March and April, were heavy from May through July and then declined rapidly to insignificant numbers by September. Winter ticks first appeared late in October, increased gradually to peak abundance in late December, then declined to insignificance by March. The spinose ear tick was present throughout the year, with populations being moderate to high at practically every examination. Cattle lice populations were extremely low throughout the year.

Horn flies appeared in April, increased steadily to moderate numbers by June, remained at this level until late September, and disappeared with cool weather during late October and early November. Light to moderate grub infestations were present in the back of cattle from early January until mid-March. Some transmission continued to occur in the infected herd but the segregated herd remained clean. It was thus demonstrated that young calves removed from the presence of carrier cows could be maintained anaplasmosis free. The test was discontinued this year.

In Mississippi studies on the relative importance of day-and night-feeding insects in the transmission of anaplasmosis, which were initiated in 1963, were continued in 1964. In the main test, three groups containing negative, splenectomized steers were used, one group being exposed continuously, one during the day only, and one at night only. The test period of 6 weeks (June 1 - July 13) was divided into three 2-week intervals and during each interval one other group of cattle of the same composition was exposed continuously in hopes of pinpointing the 2-week period in which most transmission occurred. Each group was exposed daily with animals infested with anaplasmosis.

During the first two weeks (June 1-13) horse flies were moderately abundant, but declined in numbers abruptly and only small numbers were present the last 3 weeks of the test. Populations of horn flies were relatively low and stable flies relatively high throughout the test period. Mosquitoes were fairly numerous the first week of the test but populations declined thereafter and remained low throughout June. Populations rose rapidly early in July and were high during the last two weeks of the test. The mosquito population was largely Psorophora confinnis until the last week of the test, when Anopheles quadrimaculatus increased abruptly to make up about 40 percent of the population.

In the main 6-weeks test, two cases of anaplasmosis developed in each of the continuous- and day-exposure groups, but none occurred in the night-exposure group. In the other groups exposed continuously for 2 weeks, one case each occurred in the first (June 1-16) and second (June 16-29) exposure periods but none developed in the third (June 29-July 13). These results indicate that anaplasmosis transmission most likely occurred during June when horse flies were most abundant. This indication is supported by the fact that no transmission occurred in the cattle exposed at night when only mosquitoes attacked them. However, additional studies are needed to clarify the role of mosquitoes as possible vectors and to determine whether one or more species of horse flies is capable of transmitting the disease.

At Beltsville, Md., studies on the transmission of bovine anaplasmosis and the development of the disease organism in experimental vectors were continued in cooperation with personnel of the Animal Disease and Parasite Research Division.

Efforts to colonize a "Nevada line" of Dermacentor andersoni were unsuccessful. The larval progeny from a single engorged female received from Nevada did not attach and feed on the test calf, and all died.

An adult-to-larva hereditary transmission trial with Dermacentor occidentalis was negative. Several thousand larvae attached and fed on the test calf, but anaplasmosis was not transmitted. The calf was proved susceptible by inoculation with Anaplasma infected blood.

Studies were conducted with Dermacentor occidentalis to determine whether or not the ticks can become adapted to infection with Anaplasma marginale by feeding one or two of the developmental stages in each generation on calves acutely infected with A. marginale. The plan of study was to feed adults on infected calves, feed the resulting larval progeny on guinea pigs and then test part of the ensuing nymphs on a susceptible calf. If transmission did not occur, the remaining nymphs and succeeding adults were to be fed on acutely infected calves and tested again in the following generation. In the first generation studies the adult-to-nymph trial was negative and the test calf proved susceptible by challenge. Approximately 1,000 nymphs were allowed to feed on an infected calf and 50 of the resulting adults were tested on a susceptible calf for a trans-stadial transmission. This experiment has not yet been concluded. The remainder of the D. occidentalis adults, infected as nymphs will be fed again on an infected calf and tested again as 2nd generation nymphs.

Systematic studies on Anaplasma-infected and non-infected D. occidentalis salivary glands are being conducted concurrently with each transmission experiment.

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AREA NO. 15. SHEEP AND GOAT INSECTS

Problem. Sheep and goats are attacked by a variety of insects and ticks that are responsible for losses of many millions of dollars annually in reduced weight gains, decreased production and quality of wool, and in deaths of animals from gross attacks and insect-borne diseases. Sheep keds are a particularly serious pest in the northern States and screw-worms in the southwestern States. Fleeceworms, lice, and ticks are important pests wherever sheep and goats are raised. Safer, more effective, nonresidue-forming insecticides are needed to combat these pests. There is a special need to develop systemic insecticides that when given at low levels in feed, salt, or water would effectively control pests of sheep and goats and thereby save growers the expense of rounding up and treating flocks several times a year. New approaches to control, including attractants, chemosterilants, and radiation, should be explored and developed for controlling certain pests, as was done for the screw-worm in the Southeast. The possibilities of controlling insect pests of sheep and goats with insect pathogens, parasites, and predators also need to be investigated. Additional basic studies on the biology of the insects involved are essential for the development of biological and sanitation measures for their control. Research is urgently needed to determine which insects other than sand flies transmit bluetongue and the role of insects and ticks in the spread of other diseases of sheep and goats.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving basic and applied research on insects and ticks which affect the health and productivity of sheep and goats. Studies are conducted on the biology, physiology and nutrition of pests of sheep and goats, particularly the screw-worm and Culicoides gnats, with some attention to sheep keds and lice; on the nature of resistance to insecticides and on the length of time insecticides remain on animal skin and hair; and on the absorption, metabolism, degradation, excretion, and mechanism of action of insecticides on the insects. A program is underway to find new ways to control pests of sheep and goats, with special emphasis on chemosterilants, antimetabolites, attractants, and non-insecticidal materials. Efforts are being made to develop adult screw-worm attractants for determining the abundance of natural populations and for use in baits for control. Research is concerned with the development of more effective contact and systemic insecticides and with studies to devise sanitation or management procedures to minimize or prevent insect reproduction. Primary emphasis is given to the evaluation of new materials that leave small amounts of or no residues and to testing of formulations that will prolong effectiveness against insects and minimize toxicity hazards. Studies are conducted in cooperation with the Animal Disease and Parasite Research Division to determine the occurrence of residues in tissues of animals

treated with insecticides. A limited program is being conducted on the relationship of insects to diseases of sheep and goats, involving experimental transmission from diseased to healthy animals with various species of insects, and insect surveys in epidemic areas. Current studies are centered on the insect vectors of bluetongue disease of sheep in cooperation with the Animal Disease and Parasite Research Division. The research is conducted in major laboratories in Kerrville, Tex., and Corvallis, Oreg., and in satellite laboratories in Mission, Tex., and Denver, Colo.

The Federal scientific effort devoted to research in this area totals 4.9 professional man-years. Of this number, 1.9 is devoted to basic biology, physiology, and nutrition; 1.4 to insecticidal and sanitation control; 0.4 to insecticide residue determinations; 0.4 to insect sterility, attractants, and other new approaches to control; 0.6 to insect vectors of diseases; and 0.2 to program leadership.

PROGRAM OF STATE EXPERIMENT STATIONS

There is a limited program in the States on insects affecting sheep and goats. Research in progress on the control of the sheep nose bot and the sheep ked is providing information useful to the livestock industry. The association of sheep nose bots with disease incidence is being examined. Various new insecticides are being administered to determine their effectiveness in control. Studies of application methods are being performed to obtain more satisfactory results with reduced labor cost and increased treatment safety. General insect pests affecting other livestock as well as sheep and goats are receiving careful attention. Results of studies of the biologies and control of lice and various fly pests are applicable in most cases to all hosts.

There are 0.9 professional man-years dedicated by the States to research exclusively on sheep and goat insects.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Screw-worm. Research continued on the screw-worm fly at Mission, Texas, in support of Southwestern screw-worm eradication program. Special studies were continued to develop a strain of flies resistant to starvation. Continuous selections resulted in a gradual increase in resistance to starvation until only negligible mortalities occurred in 72 hours in the 19th generation, in 96 hours in the 36th generation and in 144 hours in the 40th generation. Substrains removed from selection in the 18th and 30th generation retained their ability to withstand starvation. The sexual vigor of starvation resistant flies decreased gradually as resistance increased but the substrain removed from selection showed almost normal vigor 7 and 9 generations later. When females of the 27th generation of the

resistant strain were held with males of the same strain under 96 hours of starvation no viable eggs were produced. When females were fed the hatch of eggs was 38 % as compared to only 14% when only males were fed. When both sexes were fed hatch was 57% or about the same as that for fed unselected females and males.

Studies were continued on the mating behavior. It has been generally believed that female screw-worm flies mate only once, however, close observations showed that a high percentage of the female flies that mated with 1-day old males (immature) mated a second time with mature males. On the other hand only 2 of 110 females first mated to mature males remated, 1 willingly and the other under duress. Egg viability was only 24% when females were mated with 1-day old males but increased to 65% when females were remated with mature males. When 1-day old males were exposed for 8 hours with 3-day old (mature) females and then replaced with mature males egg hatch was only 52%, compared to 89% for matings of mature males and females. These results indicate that females seldom remate if their first mating is satisfactory, i.e. with a mature male.

Competitiveness of irradiated (R) and non-irradiated (N) males was compared in multiple mating of females. Frequency of remating was increased by subjecting females to starvation periods of 20 to 24 hours and all matings were confirmed by observation. Females mated first with R males and then with N males averaged 33% to 48% fertility in 2 tests, compared with 75% to 85% for the controls. However, in the reciprocal matings fertility was 80% in both tests, indicating that R males were not competitive with N males. Mortality and fecundity of starved controls subjected to single matings were normal; however, 33% to 71% of the remated females failed to survive. Actual cause of death following forced second matings has not been determined but these observations help to clarify SAG test results in which aggressive males greatly accelerate female mortality.

Occasionally mating and fecundity studies have been conducted in which oviposition was induced immediately after copulation. Since duration of sperm storage in some mammalian females greatly affects fertility, this possibility was investigated in screw-worms. In 2 out of 3 tests fertility ranged from 47% to 55% when oviposition occurred within 3 hours of copulation, compared with 78% to 86% at 24 hours. In a third test fertility was 59% within 4 minutes but ranged from 80% to 97% from 3 hours to 4 days. Although the influence of sperm storage on fertility has not been clearly defined, a minimum 24-hour period between copulation and oviposition has been adopted in mating and fecundity studies.

Three screw-worm males selected at random from the Florida colony mated a total of 72 females each. All matings were confirmed by observation during daily 4-hour sessions. Two males ceased mating at 7 days when excessive wing damage appeared to interfere with proper positioning, and the other was dead on the 7th day. Peak mating activity occurred during the 3rd to

5th days. Total matings ranged from 1 on the 1st day to a maximum of 20 on the 3rd day; however, from the 3rd to 6th days fertility primarily occurred only among the first 7 matings. Total females fertilized per male (hatch 1% to 98%) ranged from 18 to 24. These results are in agreement with previous studies in which mating activity was evaluated only on the basis of hatching records. Although female remating seldom occurs following copulation with a mature, spermatous male (unless the females are too weak to elude the male), almost 70% of the females in the above test remated.

Studies were conducted to compare the ovarian growth of normal and starvation-resistant female flies under starvation, feeding after starvation and continuous feeding. Under starvation very little growth occurred but when food was provided the starvation-resistant females showed faster development than those of the normal strain. Ovarian growth was comparable when the two strains were fed continuously. Ovarian growth was more rapid in females fed meat than in those on a meatless diet.

Bioclimatic studies showed that both normal and selected strains of newly emerged screw-worm flies survived well when held 30-45 hours at 32° F, mortalities being 25 and 10 percent, respectively. Oviposition and egg viability of surviving females was not affected by the exposure. Exposures of 48-72 hours at 105° F caused 46 and 38% mortalities of the two strains and oviposition by the survivors and viability of eggs was greatly reduced.

Laboratory studies showed that male screw-worm flies reared on horse meat were about 25 percent larger (based on weight) than flies reared on the liquid medium now used for mass production of screw-worms for use in the Southwestern control program. Also the meat-reared males were able to mate with 40% more females than the liquid-medium reared flies, although no difference was observed in the number of mating attempts by the two strains.

A preliminary investigation in Mexico made in January indicated that in average winters there is very little screw-worm overwintering in the northwestern corner of Sonora or in the northern part of lower California. However, in the coastal regions of Sonora between parallels 28° and 30°30' it is seldom cold enough to completely eliminate screw-worms. Instead they are confined to certain bowl-shaped terrain features locally known as "bahias." These warm, moist bahias also furnish preferred pasture for cattle both winter and summer. It appears that the bahias have somewhat the same relation to screw-worm survival as the river valleys in southwest Texas, but it is not known to what extent screw-worms move from one bahia to another at different times of the year.

In Texas field tests were conducted to study the relative dispersal abilities of irradiated normal and selected (starvation-resistant) strains of screw-worm flies. A total of 330,000 flies of each strain were distributed by airplane in two releases along a 6-mile swath on a large improved range area. Totals of 1659 selected strain flies and 2252 normal flies

were recovered from traps, indicating that the normal flies were more vigorous than those of the selected strain.

In August 1964, a test was initiated in a 2000-square mile area in Veracruz, Mexico, to determine the efficiency of dispersal of flies dropped from aircraft at 8-mile swath intervals as compared to the standard intervals of 2 to 4 miles. Eleven releases of 400 males per square mile were made between August 29 and November 6. Since the area was naturally infested with screw-worms, efficiency was based on the percentage of sterile egg masses collected on wounded animals in pens located 0.1, 2 and 4 miles from release lines as compared to that on animals in a control area. Fly traps were operated at each pen from October 19 until December 1 to obtain data on the relative abundance of wild and released flies. The number of egg masses was fairly high early in August but declined rapidly with the onset of hot-dry weather and remained fairly low until late September. The numbers of egg masses began increasing with favorable weather early in October and remained fairly high until termination of the test. A few sterile egg masses were collected after the first male release. The percent sterility increased steadly thereafter to a peak of 68 percent by October 2, and declined gradually to about 20 percent at time of the last fly drop on October 27. There were no significant differences in egg mass sterility at different distances from lines of dispersal, indicating that the flies dispersed uniformly and apparently rapidly. All trap catches showed larger numbers of sterile flies than wild flies from October 26 through November 6 but native flies outnumbered sterile flies in all traps by November 14. Only 2 sterile flies were present in collections on November 19 indicating a maximum longevity of about 3 weeks.

B. Insecticidal and Sanitation Control

1. Screw-worm. Research was continued in Texas to develop more effective insecticides for controlling screw-worms affecting livestock. Of twenty new compounds screened for larvicidal effectiveness at 10, 1.0 and 0.1 ppm in screw-worm larval medium, four were highly effective, killing all the larvae at 1.0 ppm: Shell SD-8964, Shell SD-8988, Shell SD-8967, and Geigy GS-12968. None of the compounds screened were effective at 0.1 ppm.

In field tests in Mexico, cattle infested with 1- and 2-day-old screw-worm larvae were sprayed or dipped in promising insecticides. Shell Compound 4072 in a dip or spray at 0.1% killed all the larvae, as did Cela S-1942 in a 1.0% spray. Hooker HRS-1422 as a 0.25% spray and Shell Compound 4072 as a 0.08% dip were fairly effective but permitted a few larvae to survive. Telodrin as a 0.05% spray killed both 1- and 2-day-old screw-worm larvae but it also killed 3 of the 4 cattle treated. Bayer 37289 (0.25% spray) and Bayer 38333 (0.1% spray) killed all the larvae in one test, but not in another. Sprays containing 0.1% of ethion, 0.1% of Dowco 175, 0.05% of dimetilan, 0.01% of Bayer 29952, 0.01% of Stauffer N-2790, or 0.01% of Bayer 38156 were ineffective.

Previous research has shown that sprays containing 0.1% or higher concentrations and a dip containing 0.1% of Shell Compound 4072 are effective screw-worm larvicides. In new tests in Mexico, cattle with wounds containing 1- and 2-day-old screw-worm larvae were dipped in vats containing either 0.05% or 0.1% Shell Compound 4072. At examination 24 hours after treatment, no live larvae were found in wounds on cattle dipped in 0.1%. All 1-day-old larvae were killed by 0.05%, and live 2-day-old larvae were found in only 1 of 16 wounds.

2. Lice. In previous studies in Texas, Ciodrin at 0.3% and 0.15% were highly effective against biting lice. Angora goats with one month's fleece were sprayed with a lower concentration of Ciodrin, 0.075%, 50 gallons of spray being used for the 230 goats. No live lice were seen on 10 animals checked 1 day after treatment, but 4 of 8 goats checked 1 week after treatment had live lice and 7 of 10 checked 1 month after treatment had light to moderate populations of lice. Thus, 0.075% Ciodrin is too low a concentration to control biting lice of Angora goats. In previous tests, sprays containing 0.1% Shell Compound 4072 were effective in controlling lice on Angora goats (freshly-sheared). In new tests, 99 newly-sheared goats were sprayed with 0.05% Shell Compound 4072. At 1 week, no live lice were found, but at the next shearing all goats examined were lightly infested with biting lice.

3. Sheep Nose Bots. A pour-on treatment of 4% Ruelene in oil at a dosage of 200 mg/kg was systemically effective against all three instars of Oestrus ovis larvae in nasal areas of sheep. Dosages of 100 and 50 mg/kg were completely ineffective against the second- and third- instar larvae, but completely effective against first-instar larvae. Treatments also effective against first-instar larvae were: trichlorfon, intramuscular injection at 50 mg/kg, pour-on of 8% in oil at 400 mg/kg; Famophos, intramuscular injection at 50 mg/kg; and Stauffer R-3828, drench at 100 mg/kg. Five other insecticides, including 2% coumaphos as a pour-on in oil at 50 mg/kg, failed to control any of the three instars of the nose bots.

C. Insecticide Residue Determinations

1. Residue Studies. In Texas, a technique for the analyses of tissue residues of the herbicides 2,4,5-T, propylene glycol butyl ether ester and 2,4,5-T acid has been developed and applied in a test in which 3 sheep were poisoned by the herbicides. With both compounds residues were deposited primarily as the acid or its salt. Residues deposited as the acid ranged from an average of 44 ppm in omental fat to 261 ppm in kidney with 73 ppm in muscle and 67 ppm in liver. Some residues were deposited as the ester. The highest was 1.25 ppm in kidney.

2. Toxicity Studies. Research was continued in Texas in cooperation with veterinarians of the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

Studies begun last year on effects of feeding apholate to sheep were continued. Two rams and three ewes survived 638 daily doses of 1 mg/kg. A moderate deficiency in white cells developed and persisted for 128 days after feeding of apholate was discontinued. A new chemosterilant, hempa, appeared to be considerably less toxic than apholate but it produced the same deficiency in white blood cells. A fourth ewe died after 574 daily doses.

Studies were conducted to determine the toxicity of 72 insecticides to cattle, sheep and goats. Toxicities ranged from impossibly dangerous to reasonably safe. Promising new insecticides showing low toxicities were Shell SD-8447 and Cela S-1942.

Studies were initiated on the toxicity to sheep of several insecticides employed to control crop pests. One of the more interesting findings was that sheep do not consume demeton, even when partially starved, when it is added to feed or sprayed on grass. This finding needs to be enlarged upon and firmly established for both cattle and sheep. If true, then poisoning of sheep and cattle would be most unlikely in a pasture where both treated and untreated forages are available.

In one study sheep were observed to increase their tolerance to malathion as both the dosage and frequency of administration were increased.

D. Insect Sterility, Attractants and Other New Approaches to Control

1. Screw-worm. In Texas 20 of 255 compounds screened as chemosterilants caused sterility in one or both sexes of screw-worms when administered as topical treatments or fed to adult screw-worm flies. Some of the compounds sterilized by both methods of administration; some sterilized only one sex and some sterilized both male and female flies. A review of past chemosterilant screening revealed that of the aziridine compounds screened, 92 were effective either by multiple-oral administration or topical application, or both. Only 8 were less than 100% effective when administered orally. In the future, routine use of topical applications in chemosterilant screening will be dropped in favor of multiple-oral administration.

It has been shown that there is a differential susceptibility between males and females sterilized with metepa. Tests with ENT-50450 showed similar results, with the males about 9 times more susceptible than females on the basis of dosage/unit of body weight. There was also a greater variation in results obtained with females than with males. Starvation also increased the effectiveness of ENT-50450. The antifertility effects of another chemosterilant, ENT-25296, were enhanced by subjecting treated flies to periods of temperature stress (98° or 14° F), after administration of sterilizing or highly effective substerilizing dosages.

Male and female screw-worm flies can be sterilized by exposure to certain chemosterilants, but most of these chemicals adversely affect mating

activity, longevity, or vigor. Three new chemosterilants were found that equal or surpass radiation in their effectiveness in achieving sterility of screw-worms. ENT-50838 applied topically provided a wide margin of safety between the minimum toxic quantity and the sterilizing dosage. Males sterilized with this material were hypercompetitive; they were sexually more aggressive than irradiated flies. The other two compounds, ENT-50716 and ENT-50781, were more toxic to the flies, but they were approximately equal to radiation as sterilizing agents.

In Texas 154 chemicals and other materials were screened as screw-worm attractants. Of these, 22 were equal to or better than the standard liver bait and require further evaluation. Some have been tested in the field in limited tests. The most outstanding were isovaleraldehyde, ethyl isovalerate, and an ethanol extract of the flowers of Yucca treculeana. Liver-baited traps were seldom as effective as traps containing these materials. The presence of blooming wild flowers interfered with these tests. Tests made during peak blooming seasons usually gave negative results.

The presence of a pheromone produced by males that is attractive to virgin female screw-worm flies was confirmed. In Texas, by means of the cold-trap method, 3500 ml of condensate were collected over a 5 1/2-month period from a cage containing virgin male screw-worm flies. Benzene and chloroform extracts of the condensate were capable of changing the behavioral pattern of virgin female flies. Sexually mature females in the presence of the odor go through "searching" motions and finally behave as in an aggressive mating "strike". Young (1-2 days old) females gave little or no response to the male odor, but 3-day-old females exhibited a definite activity, including the imitation of "male-type strike". Four-day-old females reverted to the response of 1-day-old females, but activity increased again in 5-day-old females. The greatest response was observed in 6-day-old females, with 7- and 8-day-old females showing a decrease in total number of "strikes". This decrease may have been due to wing damage, normal at this age. Other studies are in progress, including fractionation of the extracts to find the effective material. Another extract, made by filtering the air in the eradication colony room, brought a response on the part of both males and females.

E. Insect Vectors of Diseases

1. Biting Flies and Gnats. Studies were continued in cooperation with the Denver, Colo., laboratory of the Animal Disease and Parasite Research Division, on the transmission of bluetongue disease of sheep. When the virus of bluetongue disease was injected into the hemocoel of Culicoides variipennis gnats by intrathoracic puncture, it increased in titer each day for 6 days. After a peak was reached at about 6 days, there was no appreciable loss in titer of gnats held as long as 28 days. Infected gnats transmitted the virus to embryonating chicken eggs by feeding either on blood or allantoic fluid, but transmission was uncertain if the gnat fed only on allantoic fluid. It is now possible to assay the infection and infectivity of insect material without using sheep. Tissue culture assay also indicated

that bluetongue virus may multiply 1,000 to 10,000-fold in Culicoides variipennis; the multiplication occurred in gnats inoculated with virus solution. This multiplication also occurred sporadically in the gnats infected by feeding on sheep. In gnats inoculated with an egg-adapted virus, 100% of the gnats became infective to chick embryos 2 to 4 days after inoculation and 1 bite was sufficient for transmission. In trials with sheep, 2 bites were sufficient to give transmission. With small numbers of bites, sheep-to-cow, cow-to-cow, and cow-to-sheep transmission has been demonstrated.

In a study area near Hudson, Colo., where bluetongue was epidemic in sheep in 1963, an epidemiological study was conducted in 1964. The results indicated that Culicoides variipennis and Culicoides spp. were more likely to go to sheep for a blood meal than were mosquitoes, Liptoconops, Tabanidae (horse and deer flies) and Simuliidae (black flies).

Previous studies have not indicated the mosquito, Culex tarsalis, capable of transmission of bluetongue. In further tests, this mosquito was injected (intrathoracically) with bluetongue virus; after a 10-day "incubation" period in the mosquito, the virus was isolated. The BT-8 strain of bluetongue was used; similar isolations were earlier made following a 43-day insect "incubation" period using the strains BT-8 and BT-262.

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AREA NO. 16. POULTRY INSECTS

Problem. Numerous species of insects, mites, and ticks are common pests of poultry throughout the country and if not controlled can make poultry raising unprofitable. They cause poultry to look unsightly, reduce weight gains and egg production, and mar the skin, which results in downgrading of quality and lower market prices. Pests such as black flies and mosquitoes transmit leucocytozoon and fowl pox disease which exact a heavy toll in death and unthrifty poultry each year. House flies spread parasites and enteric diseases which may decimate flocks. Safer, more effective nonresidue-forming insecticides are needed to combat these poultry pests and vectors of diseases of poultry. Better materials are needed for direct application to poultry or in poultry houses to control lice, mites, and ticks and for use as larvicides or fly baits to control flies. Materials are especially needed which, when given in feed or water, would act systemically to control external pests and render droppings toxic to fly larvae. Exploratory studies are needed to investigate possibilities of developing attractants, chemosterilants, antimetabolites, or other new methods of combatting poultry pests. Biological and sanitation methods of control offer excellent possibilities for control and need to be emphasized. There is a special need to investigate the roles of insects, ticks, and mites in the transmission of poultry diseases.

USDA AND COOPERATIVE PROGRAM

A continuing study is underway in the Department involving basic and applied research on insects, mites, and ticks that affect the health and productivity of poultry. Studies are designed to determine breeding habits and reproductive capacities of various poultry pests and to gain further knowledge on the nature of resistance of these pests to certain insecticides. Work at present is devoted mostly to lice and the northern fowl mite, and to the house fly, which breeds abundantly in poultry droppings. A newly expanded program aims to find new ways to control pests of poultry with special emphasis on use of chemosterilants, antimetabolites, attractants, and noninsecticidal materials and methods. Current studies in this field are largely limited to house flies. They include investigations of physical and mechanical methods for controlling house flies being conducted in cooperation with the Agricultural Engineering and Animal Husbandry Research Divisions.

Research is concerned with the development of more effective insecticides for the control of poultry pests. New chemicals are screened in the laboratory for contact and residual toxicity to lice and mites attacking poultry and to house flies, and promising ones are tested for effectiveness under practical field conditions. New methods of utilizing insecticides more efficiently and safely are being investigated, with special attention to finding materials that, when given orally in water or feed, will act systemically to kill lice and mites on the poultry, and render the droppings toxic

to fly larvae. Efforts are also being given to methods of sanitation and management to control breeding in accumulations of manure in poultry houses. Studies are conducted to determine the occurrences of residues in tissues of poultry treated with insecticides. Work is done in cooperation with State Experiment Stations and poultry raisers at Gainesville, Fla., Stoneville, Miss., Corvallis, Oreg., and Kerrville, Tex. Additional research is conducted at Lake Charles, La. Research is conducted at the University of California at Berkeley and the University of Georgia under grant support.

The Federal scientific effort devoted to research in this area totals 2.2 professional man-years. Of this number, 0.4 is devoted to basic biology, physiology, and nutrition; 0.9 to insecticidal and sanitation control; 0.2 to insecticide residue determination; 0.6 to insect sterility, attractants and other new approaches to control; and 0.1 to program leadership.

The Federal support devoted to research in this area under grant support totals 0.4 man-years. Of this number 0.1 is devoted to basic biology, physiology and nutrition and 0.3 to biological control.

PROGRAM OF STATE EXPERIMENT STATIONS

The States are performing both basic and applied research on poultry insects. Studies are in progress to determine the distribution and amount of damage caused by the more than 50 external parasites of poultry in the various states. As the extent of injury is determined, harmful species are studied to obtain information on their life histories and ecology. For example, in the northern fowl mite, the rate of population increase, the incubation periods of the eggs and duration of the immature stages are being studied. The effects of various population levels in production and fertility of eggs are also under investigation. The development of laboratory rearing methods is a pre-requisite for much of this research.

Control studies include comparisons of insecticides. New materials are constantly being evaluated as replacements for chemicals to which some poultry pests have become resistant. Treatment methods range from feed additives, which prevent fly development in manure or provide systemic control of parasites on the birds to direct or area applications. Insecticide residue analyses are performed to determine concentrations of parent compounds and metabolic products at specific intervals following applications. Rates of degradation are determined by bioassay and chemical analyses in eggs and tissues of the birds.

Studies are in progress on the effects of insecticides and management practices on the natural enemies of pest flies. Cultural practices which favor the biological control agents are integrated with selective applications of insecticides to minimize or prevent the destruction of natural enemies, but still obtain maximum kills of pest flies.

Research on external parasites of poultry also includes detailed studies to identify vectors of diseases. Suspect vectors are reared and fed on infected birds and transferred to healthy ones. Life history studies are performed on many of these insects.

There are 6.0 professional man-years committed by the States to research on poultry insects.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies were continued on the biology of Anopheles quadrimaculatus by artifically augmenting an isolated breeding area. It was shown that (1) The density of the population was increased in the study area when a constant breeding area was supplied to egg-laying females and when a blood source was provided, (2) Wild females preferred natural ponds as an egg-laying site but used artificial ponds when natural areas were not present, and (3) Anopheles quadrimaculatus adults preferred resting boxes which were painted black and placed on a horizontal plane.

New rearing diets and rearing techniques were evaluated for Anopheles quadrimaculatus. Rye grass infusion or extract accelerated larval development. There was a positive correlation between the number of larvae per rearing pan and larval mortality. Protozoans were an important source of food for the larvae. A rearing method incorporating all of these factors increased survival and size of the insects and decreased the time required for development and their tolerance to insecticides. Similar results were obtained with four other species of mosquitoes, Aedes aegypti, A. taeniorhynchus, Culex quinquefasciatus, and Aedes triseriatus.

A new method of separating pupae of Anopheles quadrimaculatus from larvae has been adopted. When mixtures of both are placed in ice-water, larvae sink and pupae float allowing rapid separation through the use of a funnel. Time required to separate the stages in colony production was reduced 86%.

At Corvallis, Oreg., studies were continued on the biology of the mosquito, Aedes increpitus in the Willamette Valley. During the winter months of the last three years, larvae of this species have been collected in numerous habitats of the flood plain of the Willamette River. Following an unusually protracted period of subfreezing temperatures during which a low of 8°F was registered and near-record floods occurred during which all low lying areas in the Willamette Valley were inundated for several days, larvae could be readily collected. The strain of increpitus in the Willamette Valley apparently has become well adapted physiologically to the rigors of the area over a long period of time. In other studies, a strain of Culex pipiens quinquefasciatus, which is orange in color as 4th-instar larvae and newly formed pupae, has been isolated. The strain has bred true for three generations and appears to be genetically recessive.

At Lake Charles, La., basic studies on the biology of floodwater mosquitoes was conducted. Studies have shown the comparative longevity, blood-feeding, and oviposition patterns of different species of flood-water Aedes species. Aedes taeniorhynchus, A. sollicitans, and A. infirmatus are more important as pest mosquitoes than other species that occur in the area. Studies on the amount of blood ingested by 12 pest mosquito species occurring in Southwestern Louisiana indicated that females of all species ingested sufficient blood at one feeding to at least double their body weight. Psorophora cyanescens (Coquillet), Aedes atlanticustormentor, and Anopheles quadrimaculatus Say, more than tripled their body weight with blood from one feeding.

Research has been conducted under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the production, relative abundance and dispersal of pest mosquitoes in the Gulf Coast area of Louisiana. Data has been maintained on rainfall and temperature in relation to mosquito production and some information has been obtained on the influence of rains versus tidal action in mosquito production. An impoundment is being developed to study the effects of impounding and water management procedures on mosquito production in the area.

2. House flies. At Gainesville, Fla., research was continued on basic biology of the house fly. Evidence was obtained that an olfactory attractant, or pheromone, specific for the males of Musca domestica L. is not produced only by females. The attractant was found on contaminated holding cylinders and on dead and non-virgin females. In addition, live males were also somewhat attractive. The degree of attraction was of a low order, resembling other reported sex pheromones of the house fly. This attractant was soluble in methane and slightly soluble in benzene. Data also showed that the time of day at which pupal eclosion occurs is influenced by photoperiod, but photoperiod may not be the only controlling factor. Response to insecticides was also shown to be regulated by photoperiod.

In mating experiments, female house flies mated more readily with males from their own strain than with those of other strains. When normal females from the laboratory or Grand Turk (wild) strains were confined with normal males from one strain and chemosterilized males from the other, they mated more readily with males of their own strain, whether sterilized or not. Chemosterilized males competed more successfully than normal males of the same strain.

At Corvallis, Oreg., research was conducted on the genetics and physiology of house flies and data developed in these studies were used to illucidate mechanisms of insect resistance to insecticides.

Several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. Several of the mutant strains have been defined genetically and are being maintained. Most mutants

involved wing form, wing positioning, or pattern of wing venation. Three established mutant strains, classic wing, stubby wing, and dot vein have proved useful in the genetic analysis of insecticide resistance. For example two DDT resistant strains of house flies were found to possess a fifth chromosomal dominant which confers moderate resistance to DDT, but does not confer resistance to o-chloro DDT. In addition, one of the strains possessed a second chromosomal recessive which conferred moderate resistance to DDT and high tolerance to o-chloro DDT. The presence of both factors in a strain confers virtual immunity to DDT. Substrains were isolated, each possessing only one major factor for resistance and the nature of the two independent factors for resistance confirmed through appropriate crosses and bioassays. Resistance associated with the fifth chromosomal dominant is that for DDT dehydrochlorinase. The mechanism of resistance associated with the second chromosomal recessive is unknown, but apparently does not involve dehydrochlorination.

Physiological studies of mechanisms of resistance to organophosphorus insecticides in house flies showed that blocking of Ali-E with a selective inhibitor increased the accumulation of paraoxon and also the toxicity of parathion and paroxon in both susceptible and parathion-resistant strains. These results indicated that Ali-E is an important detoxifying enzyme. The role of Ali-E in organophosphate poisoning appeared to be related more to detoxication of paraoxon than of parathion in both susceptible and parathion-resistant house flies.

A resistance factor (esterase(s)) for parathion in house flies was shown to be transmitted in a dominant manner and was at least 1000 times less sensitive to inhibition by paraxon than a corresponding esterase present in a susceptible marker strain.

In other studies house flies kept in complete darkness from the time of emergence from pupae to separation of the sexes as adults mated successfully. Sex ratios were normal among the offspring.

In Oregon extensive studies were continued on the biology of the little house fly (Fannia canicularis). Investigations showed that unmated Fannia would deposit a few normal-appearing eggs, but none hatched. Most of the eggs were laid after the flies were three to four weeks old, while oviposition in the mated flies began in eight or nine days.

A standard CSMA medium used to rear the little house fly was treated with a P_{32}-labeled H_3PO_4 solution, the fly larvae being transferred to the treated medium when they reached the third larval stage. Larvae, pupae, puparia, and adults were well labeled when exposed in media containing 1 µc/larva, but not at 0.1 µc/larva. Much higher levels of radioisotope labelling were obtained at 5 and 10 µc/larva, but the 10 µc/larva medium caused undue mortality.

3. Lice and Mites. In Oregon, studies showed that colonies of the northern fowl mites on white leghorn chickens declined during early summer each year, suggesting that the mites aestivate during the warmer months. Studies to confirm this failed as mite populations also declined on chickens held at 50° to 66°F. with 10 hours of illumination.

Studies were initiated in Oregon to determine the susceptibility of Japanese quail to poultry ectoparasites. This quail is now being used in research at numerous government, industrial, and academic laboratories as an experimental animal. Quail were exposed to heavy numbers of the northern fowl mite for four weeks, but infestations failed to develop. Similar results were obtained with chicken body lice (Menacanthus stramineum) over the 4-week period.

B. Insecticidal and Sanitation Control

1. Mosquitoes. At Gainesville, Fla., the developmental program on insecticidal compounds for control of mosquitoes was continued. A large number of new candidate materials were tested in the laboratory for their potential as larvicides and adulticides; many of which proved promising for further development.

Tests were conducted to evaluate fogs of naled, fenthion, Bayer 39007, and malathion against caged salt-marsh mosquito adults. Bayer 39007 was the most effective in these tests followed by fenthion, naled, and malathion. A field test in which different formulations of malathion were applied by airplane at a ratio of 0.05 lb/acre showed a reduction of 81% in population levels with the fog oil formulation, 76% with fuel-oil formulation, and 44% with water emulsions. Airplane spray tests with four organophosphorus insecticides on adult salt-marsh mosquitoes indicated all were highly effective at low dosage rates. Comparison of the effectiveness of aerial sprays of malathion applied as a thermal fog and as a fuel oil spray showed the latter to be more effective.

Tests were conducted in the rice-growing area near Stuttgart, Ark., to evaluate the residual effectiveness of some new insecticides against natural infestations of Anopheles quadrimaculatus. The insecticides were applied to the walls and ceilings of farm buildings at 200 mg/ft^2 as wettable powders, or emulsions or both. Pre- and post-treatment counts were made of the mosquitoes resting in the treated buildings as well as in six untreated buildings which were utilized as checks. An emulsion of Hercules 9485 was highly effective, causing 99.7 to 100% reductions for at least seven weeks. As this compound was not available at the beginning of the series, it was applied from two to three weeks after the other treatments. A wettable powder formulation of Shell SD-8530 caused reductions of 98 to 100% for 9 to 10 weeks. A malathion wettable powder used as a standard caused 100% reductions for four weeks and 96 to 100% reductions throughout the 9-week test period. Hercules 9326 emulsion caused 100% reduction of the mosquito infestations for at least 9 to 10 weeks in two buildings but

in a storage shed produced only 91% to 96% control in the sixth and eighth weeks. Wettable powders of CELA S-1942 and CELA S-2225 were slightly less effective, with control falling below 70% in some buildings by the sixth week. Shell SD-8211 was highly effective in two buildings but not in a third.

Tests were also conducted to evaluate the residual effectiveness of treated cheesecloth when applied to the walls and ceiling of buildings in the same area. The cheesecloth, which was purchased in rolls 3 feet wide, was first flameproofed and then impregnated with Bayer 39007. Buildings in which a complete coverage of treated cheesecloth had been used showed 100% reduction of the mosquito populations for the full 10-week duration of the test. All buildings treated by means of a strip of cloth around the edge of the ceiling and in the corners showed 100% reduction for five weeks, and 82 to 99% control for the next five weeks. Buildings treated by means of cloth around the edge of the ceiling only, or in the corners only, showed 98 to 100% reduction of mosquitoes for five weeks, and 84 to 100% control for the next five weeks.

At Corvallis, Oreg., tests were continued on the development of more effective insecticides for mosquito control. In field tests against snow-water Aedes mosquito larvae, excellent results were obtained with lindane, BHC, and fenthion at 0.05-0.1 lbs/acre. Abate and Dursban were generally less effective. Against mosquito breeding in log ponds, granular formulations of fenthion and abate gave excellent control. Both were also effective when applied with a pump oil can. In cooperative tests in California low volume airplane sprays of malathion and fenthion showed considerable promise as mosquito larvicides.

Infusions and hot water extracts made from several tree species were tested for toxicity against Culex tarsalis larvae. Toxic elements were found in Western red cedar, ponderosa pine, and to a less extent in lodgepole pine and redwood. Similar hot water extracts made from Douglas fir, Sitka spruce, Western hemlock, big leaf maple, red alder, and white fir were nontoxic to larvae. Studies are in progress to characterize the toxic principles through fractionation of extracts.

None of seven analogs of DDT showed promise against resistant Culex tarsalis larvae.

At Corvallis, Oreg., experiments with C^{14}-TDE indicated that both susceptible and DDT-resistant Culex tarsalis larvae detoxified TDE by dehydrochlorinative and oxidative routes. The results suggested that resistance to DDT and related compounds in tarsalis involves a mechanism other than dehydrochlorination.

Studies were continued in the search for compounds that would act as synergists to overcome insecticide resistance in mosquitoes. Of a number

of phosphorus esters, butyl-containing esters were most effective although other types showed activity.

2. House fly. At Gainesville, Fla., research was conducted on the development of safer, more effective insecticides. Materials were evaluated in the laboratory as contact sprays and residual toxicants as a basis for selecting promising insecticides for field evaluations. Evaluations as residual toxicants included different formulations of the materials. Twenty-one promising compounds were tested as house fly larvicides in manure under caged poultry. Four compounds were highly effective as larvicides.

Residual tests were conducted with emulsions of malathion, diazinon, ronnel, dimethoate, naled, fenthion, and Bayer 41831, and with wettable powders of malathion and Mobil MC-A-600 against house flies in barns. All were applied at 100 mg/ft.^{2} Control was considered satisfactory as long as the reduction produced by the chemical was 75% or above. Dimethoate residues gave satisfactory control on most occasions for 14 days, after which they were ineffective. Mobil MC-A-600 wettable powder gave satisfactory control for 14 days in one test, but failed as early as the first day in a replication of the test. Other compounds gave satisfactory control for shorter periods.

At Corvallis, Oreg., research was continued to find compounds effective in synergizing organophosphorus insecticides and resistant strains of house flies. A number of different types of phosphorus esters were effective when combined with either malathion or parathion in overcoming resistance in house flies to these two compounds. Materials synergizing malathion against resistant insects differed considerably from those known to potentiate the toxicity of malathion to mice or to cause ataxia in poultry.

In Texas, dichlorvos impregnated resin strips were tested in poultry houses to determine whether control of the house fly could be obtained in this manner. Each of two 10 x 10 ft. poultry houses contained 16 white leghorn layers. Both had windows on two sides to provide cross-ventilation. The windows were kept closed in one of the poultry houses; in the other, both windows were open the first two weeks, then one was closed for two weeks, and finally both windows were closed for the final six weeks of the test. Poultry manure had been allowed to accumulate for two weeks prior to the tests. In the poultry house with both windows closed, two resin strips, containing 20% of dichlorvos were placed, one at each end of the layer unit, about four inches below the end. In the poultry house with windows opened for a part of the test, two similar strips were placed about 12 inches above each end of the layer unit. In the closed poultry house, 100% elimination of house flies was obtained in about 10 days; control of the flies remained at the 100% level for the next eight weeks. In the other poultry house there was no measurable control of flies. In a different test, 2 pounds of 4.4% Zytron granules per 100 ft^2 of manure under caged layer failed to reduce house flies more than the first two or three days after treatment. Larvae were present in the manure at the time of test, and the manure had been allowed to accumulate for two weeks.

Wind tunnel tests in Oregon showed adult little house flies (Fannia canicularis) to be susceptible to malathion, parathion, and two commercial candidate insecticides (Mobil MC A-600 and Shell SD-8436), but not to carbamate or DDT at the concentrations tested (up to 1.0% and 3.0%, respectively). Parathion and Shell SD-8436 were most effective, killing half of the flies at a concentration of 0.1%. Topical applications of heptachlor, malathion, and ronnel indicated no increase in resistance to these compounds with either of two Oregon laboratory strains.

3. Lice and Mites. In Oregon, further tests with the Hansen strain of the northern fowl mite indicated that Shell SD-8436 and Hercules 5727 were extremely toxic to the mite. The LD-50 for the former was 0.0005% and for the latter, 0.00025%; the LD-50 for malathion is about 0.0072%.

Field tests were continued in Texas to improve methods of controlling poultry lice. Litter treatments with 5% ronnel granules at 0.5 lb/100 ft^2 of litter reduced louse populations from 62 to 83% over a 37-day period after the first day when very little reduction was evident. At 1 pound of the granules per 100 ft^2, louse reduction ranged from 91 to 98%. Two applications of the 0.5 pound dosage, applied 12 days apart, were just as effective, reducing lice over a range of 93 to 98% for the 37-day period of the test. Applied at 1 lb/100 ft^2, 2% CELA S-1942 was less effective though the control increased from 55% the third day after treatment to a maximum of 90% on the 28th, 34th, and 37th day. The most effective material, 4.4% Zytron granules, gave 100% reduction of the lice within a week. A dust containing 8% of Imidan also gave 100% reduction of the lice within a week. When the Zytron granules were used to treat under cages of caged layers at 2 lb/100 ft^2, no louse control was obtained, even when the initial dosage was repeated five weeks later. Thus, there is no fumigant effect from this insecticide. In further litter treatment tests, dimetilan, Imidan, and carbophenothion, were applied at 1 pound of 2-percent dust/100 ft^2. Over the 28-day posttreatment period, reduction with dimetilan fluctuated, but never exceeded 87%; Imidan and carbophenothion gave 100% reduction of the lice. Resin pellets containing 5% of dichlorvos, applied at 0.25 and 0.5 lb/100 ft^2 of litter was relatively ineffective in controlling lice. At 0.25 lb/100 ft^2, louse control ranged from 30 to 84% over the 28-day test period; at 1/2 pound, control ranged from 58 to 88% over the pretreatment counts. However, resin strips containing 20% of dichlorvos completely eliminated lice in a closed 10x10 ft poultry house when one strip was hung at each end of a caged layer unit. When each of the two windows (cross-ventilation) in a second caged layer house were left open for two weeks, with one window then closed for two weeks, and finally both windows closed for six weeks, no measurable louse control was obtained with these strips. The recommended malathion dip for lice contains 0.5% of malathion; two dips in 0.1% malathion, 17 days apart, completely eliminated a light infestation of lice in a small flock of white leghorn hens.

4. Fleas and ticks. Two dips, 17 days apart, in 0.1% malathion emulsion failed to control sticktight fleas in a small flock of white leghorn hens

in Texas. The house and yard were not treated and the fleas averaged over 150 per bird, before and after treatment. This is, of course, a very low concentration of malathion; the recommended concentration for dipping for control of lice and mites is 0.5% malathion. Two weeks later, the same flock was dipped in 0.5% carbaryl suspension and the poultry house floor treated with 5% carbaryl dust at 1 lb/40 ft^2. This is the dust dosage recommended for control of lice and mites, but there is no recommended carbaryl dip for poultry. In spite of this treatment, the number of fleas per bird was only reduced from 160 to 50, and the count remained 50 per bird for the next 30 days. Single treatments of 5% dichlorvos resin pellets at 0.25 and 0.5 lb/100 ft^2 of litter (sawdust) gave excellent control of sticktight fleas and fowl ticks. Before treatment, populations of sticktight fleas varied from 80 to 100 per bird; fowl ticks varied from 8 to 10 per bird. On the 28th day after treatment, numbers of fleas and ticks (larval) were less than one per bird.

C. Insecticide Residue Determinations

1. Residue studies. In Texas, studies were conducted to determine whether Zytron granules applied under the cages of chickens for fly control would cause residues to be stored in the body tissues of the chickens or in the eggs. The insecticide was applied at the rate of 2 pounds of 4.4% Zytron granules per 100 square feet. Chickens were sacrificed 1, 2, 3, and 4 weeks after treatment, then the premises were retreated and chickens sacrificed one week after the retreatment. Samples of eggs also were taken at each sampling date. No detectable residues were found in the eggs or in samples of fat, breast meat, thigh meat, skin, or liver on any of the sampling dates. (Limit of sensitivity of analysis 0.005 ppm). In a second test Zytron granules were added at the rate of 1 lb/100 ft^2 to the litter in houses where chickens were kept. Samples taken two and four weeks after this treatment showed detectable residues of Zytron ranging from 0.18 to 0.199 ppm in the fat, 0.007 to 0.133 ppm in the skin, and 0.020 to 0.025 ppm in the eggs. No residues were detected in the breast meat, thigh meat or liver.

2. Toxicity Studies. Research was conducted in Texas in cooperation with the Animal Disease and Parasite Research Division on the acute and chronic toxicity of insecticides and other chemicals.

Studies showed that the polyfunctional alkylating agents apholate, tepa, and metepa, which are insect chemosterilants, injected into the yolk sacs of developing embryos after varying periods of incubation, induced congenital abnormalities in embryos that survived to the 18th day of incubation.

Doses of each compound at 250 µg and higher per egg were lethal to 4-day old embryos. Death usually occurred within 72 hours. Each compound at 125 µg. per egg was lethal to 1- and 2-day old embryos in 72 to 96 hours but allowed 4-day old embryos to continue to develop for as long as 11

days before they died. Doses of each compound at 5.4 to 25 µg. per egg usually permitted embryos to develop. Each compound induced similar congenital abnormalities such as defects of the beak, eyes, digits, and legs; cerebral and visceral hernia; edema; growth retardation and reduced weight.

Seven insecticides were studied in 280 chickens 8 to 9 weeks of age to determine the oral toxicity. The maximum nontoxic dosages found were: coumaphos, 2.5 mg/kg, diazinon and dichlorvos, 5 mg/kg, Ciodrin and Ruelene, 100 mg/kg, and dioxathion, 250 mg/kg.

D. Biological Control

1. Mosquitoes. At Lake Charles, La., research on pathogens of mosquitoes has been conducted. Field collections throughout the area have shown infections of microsporidia in thirteen species including the genera, Culex, Aedes, Culiseta, Anopheles, and Orthopodomyia. Aedes grossbecki and Orthopodomyia signifera were new host records for a microsporidian. Spore sizes were determined and classification of the microsporidia studied. Transovarial transmission of microsporidian infections was studied in 12 species of mosquitoes and demonstrated in seven of the species.

The fungus Coelomomyces was found in field collections infecting larvae of Culex restuans, C. salinarius, Aedes vexans, A. sollicitans, and Culiseta inornata. Culex salinarius, C. restuans and Aedes sollicitans represent new host records for Coelomomyces. Field infection levels varied from very low to over 50 percent.

A polyhedral virus was reported from larvae of Aedes vexans and Psorophora ferox. Both species were previously unreported as hosts of viruses. Infection levels in the field were very low. A very lethal bacteria was collected from larvae of six mosquito species.

At Gainesville, Fla., two species of Thelohania were found in A. quadrimaculatus, one infecting the adipose tissue and the other the oenocytes. One species was found infecting the oenocytes of A. crucians larvae.

E. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., studies were continued on factors affecting the attraction of mosquitoes to their hosts and factors affecting the protection time from mosquito bites afforded by repellents. A large olfactometer was developed to study these factors as well as evaluate the efficacy of various attractant materials or factors.

The effort to develop effective space and systemic repellents was continued and slightly expanded. To date several materials show some space repellency to mosquitoes in that they prevent mosquitoes from penetrating 4-mesh

screening. Materials exhibiting some systemic repellency were found and further tests will be made on these materials.

Studies were continued at Gainesville, Fla., to evaluate materials as chemosterilants for mosquitoes and to evaluate the sterility principle of mosquito control. Tests with hempa as a chemosterilant indicated it would have little value as a residual sterilant for mosquitoes. Further selection and studies were conducted with the apholate-resistant colony of Aedes aegypti to clearly define the degree of resistance. This colony is at least 10 times as resistant to the sterilizing effects of apholate as the unselected, parent colony. Selections to increase resistance will be continued.

A sterile male release study was made in a semi-isolated area with Anopheles quadrimaculatus. This area was made more favorable by increasing the number of breeding sites and introducing additional wild stock of this mosquito to populate the area. When sterile males of wild stock were released in this area, sterility of the natural population increased from a very low degree up to 42%. When the releases were changed to sterile colony males the sterility in the natural population decreased confirming earlier work showing behavior differences between colony and wild strains in seeking out wild females. Apparently a sufficient number of males was not released to reduce the population levels of A. quadrimaculatus.

Studies were continued on the evaluation of chemosterilants for mosquitoes at Corvallis, Oreg. The chemosterilant, hempa, was not highly effective in sterilizing Culex tarsalis larvae in that rates as high as 200 ppm were required. As a residual treatment in glass jars, 10 mg of hempa per square foot sterilized adult males completely, but adult females only partially. In wind tunnel tests against adults, a concentration of 10% caused high sterility, whereas a 5% spray caused only partial sterility and none was caused at 1% or lower. Males were generally more susceptible than the females.

At Corvallis, Oreg., studies were continued on sex and ovipositional attractants for mosquitoes. Preliminary tests indicated the presence of sex attractant in Culex quinquefasciatus. Further tests did not confirm this, nor the presence of a sex attractant in Culex tarsalis. Many mosquitoes are known to choose specific types of water for oviposition. Studies have shown that odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of Culex quinquefasciatus. These odors were not attractive to females of C. tarsalis. However, log pond water itself was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of C. quinquefasciatus but not to those of C. tarsalis. Gravid females of both species were more attracted to distilled water treated with 25 ppm of furfural than to water treated with 5 or 50 ppm.

2. House Flies. At Gainesville, Fla., research was continued on the development of chemosterilants and the sterility principle of control for house flies. Several hundred new candidate compounds were evaluated in primary screening and secondary development tests and many were found to exhibit sterilizing efficacy against both males and females. Particular attention was paid to evaluating two compounds--hempa and hemel--as sterilants by several routes of administration. These two materials will sterilize both sexes of the house fly.

Two series of field tests were conducted at farms in Florida to evaluate the effectiveness of two chemosterilants, hempa and apholate, for the control of house flies. At the farm treated with hempa, house flies were reduced in abundance from 71 per grid to 0 within eight weeks. Grid counts remained at zero for the remainder of the test period. At the farm treated with apholate, population levels decreased from 200 per grid to less than 10 within six weeks and remained constant at a low level throughout the remainder of the test.

Ninety chemicals were screened as chemosterilants against adult house flies. Six compounds were toxic and 10 reduced the fertility to some extent in fly food or sugar. Thirty-two compounds, previously shown to sterilize house flies, were also tested again at higher or lower concentrations.

Tests were conducted with 21 compounds to determine their effectiveness as male house fly sterilants. Of the 14 chemicals Olin 53330 (ENT-50838), Squibb Olin 53331 (ENT-50839), Squibb Olin 53356 (ENT-50840), and Squibb Olin 53263 (ENT-50842) which sterilized at a concentration of 0.05% in the sugar diet. Sankyo Co. RES-101 (ENT-50845) induced sterility at this dosage in sugar in the first two eggings.

Basic studies were continued on the cytological effects of chemosterilants on house fly reproductive systems and previous sectioning, fixing, and staining techniques have been used to study several new chemosterilants.

Olfactometers designed by Gouck and Schreck were used to initiate a search for more effective house fly attractants. Nineteen compounds were tested, using Edamin as a standard. Beef protein concentrate was attractive to females but not to males. The other materials were not as effective as Edamin.

At Corvallis, Oreg., research was conducted on chemosterilants and attractants for the house fly and the little house fly. With the little house fly, hempa caused sterility as a residual deposit on glass at 50 mg/ft^2. However, the sterilizing dose caused some fly mortality. Higher doses were highly toxic to the adult flies; lower doses did not sterilize. When fed orally in the adult food to the little house fly, hempa was toxic at 0.25% and lethal at 1.0%. High, but incomplete sterility was caused at concentrations as low as 0.01%. Topical treatments of hempa and hemel sterilized males of the little house fly without causing mortality, but

not the females. In general treatments causing a high degree of sterility did not affect the mating competetiveness of females. Four known anti-oxidants exhibited little effect on egg production or egg hatch with the little house fly. Dosages of gamma radiation greater than 1000 r given to pupae of the little house fly prevented oviposition by emerging adults.

At Corvallis research was continued on the sex pheromone in house flies. The presence of a low titre of pheromone in extracts of pupae and young females was shown and confirmed. Higher activity of the pheromone was demonstrated in 3-day-old flies. The presence of the pheromone was shown in female flies from strains of different origin. Males of different strains reacted to extracts from females of different strains, though differences in behavior were apparent.

At Corvallis, Oreg., further studies showed that the sex pheromone in female house flies increased with the age of the flies, with the greatest increase occurring on about the third day after emergence. Comparative tests with benzene extracts of female house flies showed that high concentrations applied to pseudo flies inhibited male response. Similar inhibition occurred when normal extracts were applied to large surfaces within test chambers. Comparative tests with extracts of females with different solvents showed a much greater amount of the pheromone in hexane than in other solvent extracts. Efforts are being made to determine the chemical nature of the pheromone.

In Oregon studies were continued with the little house fly, Fannia canicularis. In mating experiments with radioactive flies (reared in a medium treated with P_{32}-labeled H_3PO_4 solution) crossed with normal flies, eggs from pairings with flies exposed to 5 µc/larva were only 18.3% fertile; those exposed to 10 µc/larva did not oviposit, and untreated flies showed 88% fertility in eggs deposited. In other tests, late stage pupae of the little house fly were irradiated in a Cobalt 60 source at levels from 1,000 r to 10,000 r. Radiation dosages of 2,500 r and above totally arrested oviposition. At 1,000 r oviposition was reduced and egg hatch was only 20% of normal.

In studies with chemosterilants in Oregon, residues of 20 mg of the new chemosterilant, hempa, per square foot were only partially effective in sterilizing the little house fly. Residues of 50 mg/ft^2 gave essentially complete sterility of adult flies exposed, but mortality of the flies was high. Male flies fed 0.1% hempa in honey-water for 4 days were fully competitive with normal males, reducing the fertility of eggs laid by females by the expected amounts in a single, preliminary test. To determine the amount of hempa and the related chemosterilant, hemel, required to sterilize the little house fly, topical treatments were made. With either compound, about 24 micrograms per fly sufficed to sterilize the males. About 96 micrograms of hempa were required to sterilize the females, a dosage that caused high mortality. Hemel could not be tried above 40 micrograms per fly, due to solubility problems; this dosage failed to

sterilize the females. Both materials sterilized the males at a dose that caused little or no mortality. The flies used weighed an average of 8 milligrams apiece. Studies are being initiated to determine whether the little house fly can develop resistance to chemosterilants. In preliminary tests, a reduced hatch resulted in flies fed 0.001% hempa (78 to 62.5% hatch). Chemosterilants break down quickly on exposure. Four antioxidant compounds, under consideration as a means of reducing this breakdown,were screened to be certain they had no sterilizing action of their own. When fed at 0.1% in honey and water, none affected egg production or egg hatch.

Tests to discover attractants for the little house fly continued in Oregon. To provide a more accurate record of the duration and frequency of visits by flies on colored strings, lengths of white twine were treated with aqueous radiophosphorus solution. A consistent level of radioactivity was not induced in flies until small amounts of sugar or honey were added to the radiophosphorus solution. When this was done and the solution standardized at 50 μc/ml, effective labeling of the flies occurred in 24 hours.

In Maryland, the effectiveness of electrocutor-grid screens placed in windows plus an indoor electrocutor trap with black light lamps for controlling fly populations was evaluated in two calf barns where large numbers of house flies and stable flies were present. Although many flies were killed by the grids, no substantial reduction of the fly population within the grid-screened barn could be measured unless all major openings were closed or screened. When the animals were allowed access to exercise lots through open doors, the fly populations in the test barn and the "check" rapidly equalized.

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AREA NO. 17. INSECTS AFFECTING MAN, HOUSEHOLDS, AND INDUSTRIAL ESTABLISHMENTS

Problem. Insects, ticks, and mites are responsible for widespread human misery and certain insects cause heavy losses of food and materials in households and industrial establishments. Many of the same or closely related insects which affect man are also important pests of livestock, thus research on insects in relation to man and to livestock is mutually advantageous. Certain arthropods are vectors of major diseases which annually cause the deaths of millions of humans. Mosquitoes, for example, transmit malaria, dengue, encephalitis, yellow fever, and filariasis. Some of these diseases, as well as other arthropod-borne diseases, occur and are potentially serious problems in the United States but most of them are of more concern in other parts of the world where troops and civilian personnel of the United States are maintained. The military agencies have for many years depended on the research competence in agriculture for answers to their military insect and insect-borne disease problems. Attacks by insects, ticks, and mites frequently interfere with farm and forest work, reduce or destroy the value of recreation areas, and even make certain areas uninhabitable. Property values are often depressed and development prevented by hordes of annoying pests. Mosquitoes, bed bugs, and fleas are frequently serious annoyances in homes. Other household insects are of economic importance in homes and industrial establishments because they damage foods, fabrics, and other materials, causing losses of millions of dollars annually. There is a great need for safe, economical insecticides and satisfactory methods for their application that could be used quickly and effectively to control local infestations or outbreaks of pests that annoy man in the field or at home, especially where there are threats of disease epidemics. Improved means for controlling mosquitoes, sand flies, gnats, the imported fire ant, and similar pests should receive particular attention. More efficient repellents are needed to protect humans, particularly when other means of control cannot be employed. Special efforts should be made to develop systemic materials which when taken orally would repel or prevent insects from biting. Sanitation, habitat management, and other noninsecticidal methods of control should be reappraised, and biological control, especially with insect pathogens, needs to be fully explored. New approaches to control including chemosterilants, antimetabolites, attractants, and radiation require intensive investigation. Studies should be undertaken on the biology, ecology, physiology, and genetics of many important pests affecting man and the household in order to find weak points in their life cycles which might be utilized to improve control efficiency.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving applied and basic research on insects affecting man, households, and industrial establishments, including mosquitoes, house flies, human lice, bed bugs, gnats, fleas, ticks, mites, and other pests of man and on cockroaches, ants, and several other pests of importance in households and industrial establishments. Research is directed toward the development of more effective insecticides and repellents and involves primary screening of chemicals in the laboratory and field evaluation of promising materials. Investigations are conducted on the nature of insect resistance to insecticides, on the mode of action of insecticides, on the effects of radiation and chemosterilants, on attractants, on factors affecting attraction of biting insects to humans, and on the factors affecting the effectiveness of repellents. Attention is also given to the development of sanitation and management procedures and to biological control methods for mosquitoes, house flies, cockroaches, and several other pests. The major portion of the program is conducted at Gainesville, Fla. The remainder of the work is done at Corvallis, Oreg., Fresno, Calif., Lake Charles, La., and Beltsville, Md. Close cooperation and evaluation of research data and needs are maintained with the Defense Department through the Armed Forces Pest Control Board concerning studies on insects important to military personnel. Research funds supporting 3 professional man-years have been transferred from the Defense Department to support research on chemosterilization of house flies, basic biology and physiology of house flies and mosquitoes and pathogens of mosquitoes. Cooperation is maintained with the World Health Organization on studies for developing new insecticides and other methods of control of insects affecting man. The World Health Organization provides financial support (1/2 professional man-year) for studies at Gainesville, Fla., on the development of residual insecticides for mosquito control. Research is conducted at the University of Southwestern Louisiana and McNeese State College and by the Florida State Board of Health and the University of California under contracts and under grants at the University of Florida.

The Federal scientific effort devoted to research in this area totals 24.2 professional man-years. Of this number 5.1 is devoted to basic biology, physiology, and nutrition; 8.8 to insecticidal and sanitation control; 1.7 to biological control; 7.1 to insect sterility, attractants and other new approaches to control; 0.5 to the evaluation of equipment for insect detection and control; and 1.0 to program leadership. In addition, Federal support devoted to research in this area under contracts and grants totals 2.1 man-years. Of this number 0.2 is devoted to basic biology, physiology, and nutrition; 1.1 to insecticidal and sanitation control; 0.2 to biological control and 0.6 to insect sterility, attractants, and other new approaches to control.

Additional research (2 professional man-years) is carried out under a grant of P.L. 480 funds (S9-ENT-3) to the Facultad de Agronomia, Universidad de la Republica, Uruguay, on "Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay."

Research has been continued in Salisbury, Southern Rhodesia, Africa, under a Participating Agency Service Agreement between the Agency for International Development and the Agricultural Research Service and a Cooperative Agreement between the Agricultural Research Service and the Agricultural Research Council of Central Africa on the feasibility of the sterile-male technique for tsetse fly control.

PROGRAM OF STATE EXPERIMENT STATIONS

There is a well-diversified research program in this area in the States. Studies of insects affecting man are concerned largely with mosquitoes, flies, midges, and gnats. In many instances, results of research on livestock provide information which may be used to reduce the incidence of pests which annoy or transmit human diseases.

Biological studies include species distribution and dissemination, feeding habits, host preferences, diapause, nature of breeding sites, and seasonal population fluctuations. Chemical control studies involve screening more effective, safer insecticides to replace those to which pests have developed resistance. Studies on attractants, repellents, baits, chemosterilants, and water and soil breeding site management are being performed. Natural enemies are being sought out and evaluated for their effectiveness preparatory to eventual increase and release if this proves feasible.

Research on household insects ranges from basic studies on the biochemistry and genetics of resistant pests to tests of new methods of control involving antimetabolites, fabric impregnation and persistant insecticides which are nontoxic to warm-blooded animals. Studies on structural pests, such as termites and wood-boring beetles include evaluation of soil pretreatments with insecticides, effects of fumigation and basic investigations of habits and environmental factors influencing the establishment of infestations.

Research on insects in industrial establishments is concerned largely with the control of stored products pests. The species responsible for grain losses are being identified and the development of populations in relation to environmental factors is being investigated. The interrelationships of insects and mold development are under investigation. Control studies include evaluations of the relative resistance of different varieties of grain to insect attack, fumigants, grain protectants, and ionizing radiation. The effectiveness of various parasites and predators is also being determined.

A total of 26.5 man-years is devoted to research in this area in the States.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Basic Biology, Physiology, and Nutrition

1. Mosquitoes. At Gainesville, Fla., studies were continued on the biology

of Anopheles quadrimaculatus by artifically augmenting an isolated breeding area. It was shown that (1) the density of the population was increased in the study area when a constant breeding area was supplied to egg-laying females and when a blood source was provided, (2) Wild females preferred natural ponds as an egg-laying site but used artificial ponds when natural areas were not present, and (3) Anopheles quadrimaculatus adults preferred resting boxes which were painted black and placed on a horizontal plane.

New rearing diets and rearing techniques were evaluated for Anopheles quadrimaculatus. Rye grass infusion or extract accelerated larval development. There was a positive correlation between the number of larvae per rearing pan and larval mortality. Protozoans were an important source of food for the larvae. A rearing method incorporating all of these factors increased survival and size of the insects and decreased the time required for development and their tolerance to insecticides. Similar results were obtained with four other species of mosquitoes, Aedes aegypti, A. taeniorhynchus, Culex quinquefasciatus, and Aedes triseriatus.

A new method of separating pupae of Anopheles quadrimaculatus from larvae has been adopted. When mixtures of both are placed in ice-water, larvae sink and pupae float allowing rapid separation through the use of a funnel. Time required to separate the stages in colony production was reduced 86%.

Laboratory studies on the decline in the male/female sex ratio of Aedes aegypti (L.) during the first 30 minutes of hatching showed a straight line regression for the ratio over time. The total curve, however, suggests that the decline in the ratio of males to females over a longer time can be more nearly approximated by a logarithmic regression. The regression of all the data combined indicated a slope of 0.0098 with the regression equation thus reading log $\hat{Y} = 0.293 - (-0.0098)(X)$.

At Corvallis, Oreg., studies were continued on the biology of the mosquito, Aedes increpitus in the Willamette Valley. During the winter months of the last three years, larvae of this species have been collected in numerous habitats of the flood plane of the Willamette River. Following an unusually protracted period of subfreezing temperatures during which a low of 8°F was registered and near-record floods during which all low lying areas in the Willamette Valley were inundated for several days, larvae could be readily collected. The strain of increpitus in the Willamette Valley apparently has become well adapted physiologically to the rigors of the area over a long period of time. In other studies, a strain of Culex pipiens quinquefasciatus, which is orange in color as 4th-instar larvae and newly formed pupae has been isolated. The strain has bred true for 3 generations and appears to be genetically recessive.

At Lake Charles, La., basic studies on the biology of floodwater mosquitoes was conducted. Studies have shown the comparative longevity, blood-feeding, and oviposition patterns of different species of floodwater Aedes species. A.

taeniorhynchus, A. sollicitans, and A. infirmatus are more important as pest mosquitoes than other species that occur in the area. Studies on the amount of blood ingested by 12 pest mosquito species occurring in Southwestern Louisiana indicated that females of all species ingested sufficient blood at one feeding to at least double their body weight. Psorophora cyanescens (Coquillet), Aedes atlanticus-tormentor, and Anopheles quadrimaculatus Say more than tripled their body weight with blood from one feeding.

Research has been conducted under two contracts at the University of Southwestern Louisiana and McNeese State College. Light trap collections have shown the production, relative abundance and dispersal of pest mosquitoes in the Gulf Coast area of Louisiana. Data has been maintained on rainfall and temperature in relation to mosquito production and some information has been obtained on the influence of rains versus tidal action in mosquito production. An impoundment is being developed to study the effects of impounding and water management procedures on mosquito production in the area.

2. House Fly. At Gainesville, Fla., research was continued on basic biology of the house fly. Evidence was obtained that an olfactory attractant, or pheromone, specific for the males of Musca domestica L. is not produced only by the females. The attractant was found on contaminated holding cylinders and on dead and non-virgin females. In addition, live males were also somewhat attractive. The degree of attraction is of a low order, resembling other reported sex pheromones of the house fly. This attractant was soluble in methane and slightly soluble in benzene. Data also showed that the time of day at which pupal eclosion occurs is influenced by photoperiod, but photoperiod may not be the only controlling factor. Response to insecticides was also shown to be regulated by photoperiod.

At Corvallis, Oreg., further studies showed that the sex pheromone in female house flies increased with the age of the flies, with the greatest increase occurring on about the third day after emergence. Comparative tests with benzene extracts of female house flies showed that high concentrations applied to pseudo flies inhibited male response. Similar inhibition occurred when normal extracts were applied to large surfaces within test chambers. Comparative tests with extracts of females in different solvents showed a much greater amount of the pheromone in hexane than in other solvent extracts. Efforts are being made to determine the chemical nature of the pheromone.

In Florida, mating experiments showed that female house flies mated more readily with males from their own strain than with those of other strains. When normal females from the laboratory or Grand Turk (wild) strains were confined with normal males from one strain and chemosterilized males from the other, they mated more readily with males of their own strain, whether sterilized or not. Chemosterilized males competed more successfully than normal males of the same strain.

At Corvallis, Oreg., research was conducted on the genetics and physiology of house flies and data developed on these studies were used to elucidate mechanisms of insect resistance to insecticides.

Several mutants of the house fly were isolated from normal and gamma-irradiated strains reared in the laboratory. Several of the mutant strains have been defined genetically and are being maintained. Most mutants involved wing form, wing positioning, or pattern of wing venation. Three established mutant strains, classic wing, stubby wing, and dot vein have proved useful in the genetic analysis of insecticide resistance. For example, two DDT resistant strains of house flies were found to possess a fifth chromosomal dominant which confers moderate resistance to DDT, but does not confer resistance to o-chloro DDT. In addition, one of the strains possessed a second chromosomal recessive which conferred moderate resistance to DDT and high tolerance to o-chloro DDT. The presence of both factors in a strain confers virtual immunity to DDT. Substrains were isolated, each possessing only one major factor for resistance and the nature of the two independent factors for resistance were confirmed through appropriate crosses and bio-assays. Resistance associated with the fifth chromosomal dominant is that for DDT dehydrochlorinase. The mechanism of resistance associated with the second chromosomal recessive is unknown, but apparently does not involve dehydrochlorination.

Physiological studies of mechanisms of resistance to organophosphorus insecticides in house flies showed that blocking of ali-esterase with a selective inhibitor increased the accumulation of paraoxon and also the toxicity of parathion and paraoxon in both susceptible and parathion-resistant strains. These results indicated that ali-esterase is an important detoxifying enzyme. The role of ali-esterase in organophosphate poisoning appeared to be related more to detoxication of paraoxon than of parathion in both susceptible and parathion-resistant house flies.

A resistance factor (esterase(s)) for parathion in house flies was shown to be transmitted in a dominant manner and was at least 1000 times less sensitive to inhibition by paraoxon than a corresponding esterase present in a susceptible marker strain.

In other studies successful mating of house flies kept in complete darkness from the time of emergence from pupae to separation of the sexes occurred. Sex ratios were normal among the offspring.

3. Stable Fly. At Gainesville, Fla., studies were continued on the dog fly, a serious pest of humans and animals along the Northwest Coast of Florida. A general outbreak of dog flies occurred during the last week of August and the first 2 weeks of September in 1964. Population counts around motels and other structures and on beaches ranged from landing rate counts of 2 to 94 flies per man per minute. Bay grass deposits in 1964 were much heavier in 1964 than in 1963. Observations indicated that the insects developed in the bay grass in about the same length of time as was required for development

in CSMA medium under laboratory conditions.

Tests indicated that the effectiveness of insecticides varied with the time of exposure after blood feeding. Other research in large outdoor cages indicated that landing rate counts of stable flies were a good index of total populations since the same percentage of flies landed on human observers when the total number of flies was varied.

At Gainesville, Florida, studies were undertaken to determine the dietary requirements of adult eye gnats. Data showed that carbohydrates are an absolute requirement for survival and when present in a sufficient concentration, viable eggs are produced. The addition of protein supplements had very little effect on the production of viable eggs. Liquid forms of diets resulted in increased survival and egg production compared with dry forms of diets.

B. Insecticidal and Sanitation Control

1. Mosquitoes. At Gainesville, Fla., the developmental program on insecticidal compounds for mosquitoes was continued. A large number of new candidate materials were tested in the laboratory for their potential as larvicides and adulticides; many of which proved promising for further development.

Studies were continued to develop effective insecticidal treatments for mosquito larvae breeding in cisterns. Several materials showed considerable promise in that they were effective over several weeks. One highly promising material, abate, of low mammalian toxicity at a concentration of 0.5 ppm produced good kill of two species of mosquitoes through 8 weeks.

Tests were conducted to evaluate fogs of naled, fenthion, Bayer 39007, and malathion against caged salt-marsh mosquito adults. Bayer 39007 was the most effective in these tests followed by fenthion, naled, and malathion. A field test in which different formulations of malathion were applied by airplane at a ratio of 0.05 lb/acre showed a reduction of 81% in population levels with the fog oil formulation, 76% with fuel-oil formulation, and 44% with water emulsions. Airplane spray tests with four organophosphorus insecticides on adult salt-marsh mosquitoes indicated all were highly effective at low dosage rates. Comparison of the effectiveness of aerial sprays of malathion applied as a thermal fog and fuel oil spray showed the latter to be more effective.

During August and September of 1964 a series of tests was conducted on the island of Okinawa to evaluate twelve insecticides as adulticides and/or larvicides against Culex tritaeniorhynchus and Culex quinquefasciatus. This testing program was part of a continuing cooperative program between military entomologists and personnel of the Gainesville laboratory. As adulticides applied from a non-thermal aerosol generator, 5 insecticides were highly effective. As larvicides in laboratory tests and field tests in rice paddies 8 compounds were highly effective and abate was extremely effective.

Tests were conducted in the rice-growing area near Stuttgart, Ark., to evaluate the residual effectiveness of some new insecticides against natural infestations of Anopheles quadrimaculatus. The insecticides were applied to the walls and ceilings of farm buildings at 200 mg/ft^2 as wettable powders and/or emulsions. Pre- and post-treatment counts were made of the mosquitoes resting in the treated buildings as well as in six untreated buildings which were utilized as checks. Eight to ten weeks after treatment most of the rice fields were drained. This condition was soon followed by a gradual decrease of the mosquito populations over the entire area and the evaluations of control were then discontinued.

An emulsion of Hercules 9485 was highly effective, causing 99.7%-100% reductions for at least seven weeks. As this compound was not available at the beginning of the series, it was applied from two to three weeks after the other treatments. A wettable powder formulation of Shell SD-8530 caused reductions of 98-100% for 9-10 weeks. A malathion wettable powder used as a standard caused 100% reductions for four weeks and 96-100% reductions of 99.0% throughout the 9-week test period. Hercules 9326 emulsion caused 100% reduction of the mosquito infestations for at least 9-10 weeks in two buildings but in a storage shed produced only 91% to 96% control the sixth and eighth weeks. Wettable powders of CELA S-1942 and CELA S-2225 were slightly less effective, with control falling below 70% in some buildings by the sixth week. Shell SD-8211 was highly effective in two buildings but not in a third.

At Gainesville, tests were also conducted to evaluate the residual effectiveness of treated cheesecloth when applied to the walls and ceiling of buildings in the same area. The cheesecloth, which was purchased in rolls 3 feet wide, was first flameproofed and then impregnated with Bayer 39007. Buildings in which a complete coverage of treated cheesecloth had been used showed 100% reduction of the mosquito populations for the full 10-week duration of the test. All buildings treated by means of a strip of cloth around the edge of the ceiling and in the corners showed 100% reduction for five weeks, and 82 to 99% control for the next five weeks. Buildings treated by means of cloth around the edge of the ceiling only, or in the corners only, showed 98 to 100% reduction of mosquitoes for five weeks, and 84 to 100% control for the next five weeks.

At Corvallis, Oreg., tests were continued on the development of more effective insecticides for mosquito control. In field tests against snow-water Aedes mosquito larvae, excellent results were obtained with lindane, BHC, and fenthion at 0.05-0.1 lbs/acre. Abate and Dursban were effective at the same or lower rates, but ronnel and SD-7438 were generally less effective. Against mosquito breeding in log ponds, granular formulations of fenthion and abate were effective in control. Both of these materials, when applied with a pump oil can, were also effective. In cooperative tests in California low volume airplane sprays of malathion and fenthion showed considerable promise as mosquito larvicides.

Infusions and hot water extracts made from several tree species were tested for toxicity against Culex tarsalis larvae. Toxic elements were found in Western red cedar, ponderosa pine, and to a lesser extent in lodgepole pine and redwood. Similar hot water extracts made from Douglas fir, Sitka spruce, Western hemlock, big leaf maple, red alder, and white fir were nontoxic to larvae. Studies are in progress to characterize the toxic principles through fractionation of extracts.

None of seven additional analogs of DDT showed promise against resistant Culex tarsalis larvae.

Of 42 compounds tested as systemics in mice against Aedes aegypti, only one (ENT-27160) showed any systemic toxicity.

At Corvallis, Oreg., experiments with C^{14}-TDE indicated that both susceptible and DDT-resistant Culex tarsalis larvae detoxified TDE by dehydrochlorinative and oxidative routes. The results suggested that resistance to DDT and related compounds in tarsalis involves a mechanism other than dehydrochlorination.

Studies were continued in the search for compounds that would act as synergists to overcome insecticide resistance in mosquitoes. Of a number of phosphorus esters, butyl containing esters were most effective although other types showed activity.

2. House Fly. At Gainesville, Fla., research was conducted on the development of safer, more effective insecticides. Materials were evaluated in the laboratory as contact sprays and residual toxicants as a basis for selecting promising insecticides for field evaluations. Evaluations as residual toxicants included different formulations of the materials. Twenty-one promising compounds were tested as house fly larvicides in manure under caged poultry. Four compounds were highly effective as larvicides.

Residual tests were conducted with emulsions of malathion, diazinon, ronnel, dimethoate, naled, fenthion, and Bayer 41831, and with wettable powders of malathion and Mobil MC-A-600 against house flies in Florida. All were applied at 100 mg/ft^2. Control was considered satisfactory as long as the reduction produced by the chemical was 75% or above. Dimethoate residues gave satisfactory control on most occasions for 14 days, after which they were ineffective. Mobil MC-A-600 wettable powder gave satisfactory control for 14 days in one test, but failed as early as the first day in a replication of the test. Other compounds gave satisfactory control for shorter periods.

Tests were conducted with 19 larvicides for the effectiveness in controlling fly larvae in outdoor privies on Grand Turk Island. Larvicides were applied only when infestations were heavy and considered ineffective when the first larvae was observed. Each test was replicated three times with both water and oil carriers at two dosages. There was little difference in control between the 100 and 200 milligram dosages and diazinon was the most effective giving complete control for periods ranging from 7 to 13 days in both water and diesel oil carriers.

At Corvallis, Oreg., research was continued to find compounds effective in synergizing organophosphorus insecticides and resistant strains of house flies. A number of different types of phosphorus esters were effective when combined with either malathion or parathion in overcoming resistance in house flies to these two compounds. Materials synergizing malathion against resistant insects differed considerably from those known to protentiate the toxicity of malathion to mice or cause ataxia in poultry.

At Corvallis, Oreg., bioassay tests with heptachlor, malathion, and ronnel against the little house fly indicated little or no change in resistance to these compounds.

3. Stable Fly. At Gainesville, Fla., chemicals were evaluated in the laboratory as potential larvicides for the control of stable flies. Approximately 150 compounds were tested by exposing larvae to these compounds when they were incorporated into the larval rearing medium. Approximately 20 of the compounds were highly effective, approximately equal in activity to a standard, Bayer 39007. Tests with calcium arsenate as a larvicide indicated that it did not compare favorably with other compounds that were evaluated. In addition approximately 120 compounds were evaluated in laboratory tests as adulticides. Some 20 compounds were highly effective as adulticides in laboratory tests. Several of the more promising adulticides were tested as fogs against caged adults under field conditions. Comparative tests of adulticides against caged insects indicated no differences in effectiveness of thermal vs. nonthermal fog applicators or between fuel-oil and water-based formulations. A contract was negotiated with the Florida State Board of Health to conduct research on insecticides for controlling natural populations of dog flies (also known as stable flies) under conditions found in the Gulf Coast area of Northwestern Florida. The research contract will take advantage of research conducted at the Gainesville laboratory and evaluate insecticides under practical field conditions.

4. Cockroaches.

At Gainesville, Fla., research was continued on the development of new, more effective insecticide treatments for the control of cockroaches. In standardized laboratory tests approximately 65 new or promising candidate insecticides were evaluated as contact sprays or residual treatments against German cockroaches. The better compounds will be evaluated under natural environments.

Laboratory studies were conducted with organic phosphorus insecticides in an effort to determine the effect of various types of surfaces on the residual persistence of these toxicants. The organic phosphorus insecticides evaluated in this study (diazinon, fenthion, malathion, and ronnel) were completely ineffective on all surfaces after aging for one week. On concrete, diazinon gave 55% mortality as a 2-hour-old residue, but was ineffective after aging for 24 hours, whereas fenthion, malathion, and ronnel failed to cause mortality in the initial test. Diazinon was ineffective

initially on plaster, as were the remaining organophosphorus compounds on plaster, latex paint on concrete, and latex paint on plasterboard. On plasterboard, diazinon and fenthion produced mortalities of 100% after aging 2 and 24 hours, but were ineffective after 1 week. After aging for 2 hours, 24 hours, and 1 week on plasterboard the malathion residues gave mortalities of 55%, 80%, and 0%, respectively, whereas ronnel gave 100% mortality initially but failed to kill thereafter. Diazinon on painted concrete and painted plasterboard produced 100% kill as a 2-hour-old residue, but killed 0% and 85%, respectively, after 24 hours of aging and was completely ineffective at 1 week.

A paste bait formulation consisting of peanut butter, peanut oil, and 0.125% of Kepone was evaluated in a field experiment against natural infestations of German cockroaches (Blattella germanica (L.)). A 94% reduction of live cockroaches was achieved after the first week of the experiment and by the end of the fourth and eighth weeks, the reduction had increased to 99%. After 13 weeks the experimental area was examined very thoroughly for the presence of live cockroaches and it became evident that 100% reduction had been attained. Inspection of the area 22 weeks after treatment indicated that 100% reduction continued to be in effect. The results reported herein indicate that the paste formulation of Kepone is more effective than the pelleted formulation, which did not exceed 99% reduction during a 32-week test period.

Laboratory tests demonstrated the first confirmed case of malathion resistance in a wild strain of German cockroaches.

5. Body Lice. Tests were continued at Gainesville to develop new and more effective insecticidal powders for the control of body lice. Approximately 100 new compounds were evaluated. Approximately 36 of these were highly effective in killing lice over a one-month period and 18 were highly effective knock-down agents. Secondary screening tests have been conducted with the promising materials. Research has been initiated to find compounds that will synergize the effectiveness of organophosphorus toxicants to body lice. New, highly effective synergists have not been found to date. Sulfoxide was slightly more effective than piperonyl butoxide in synergizing the effectiveness of carbaryl against body lice.

Six colonies of body lice were maintained to study insecticide resistance in body lice. In one of these colonies which has been selected with carbaryl for 55 generations, resistance to DDT and carbaryl increased markedly. After selection of another colony with malathion, DDT-resistance was high, but lice from this colony were still as susceptible to malathion as before selection. Another colony showed decreased resistance to DDT and lindane after a year.

The search for systemic insecticides or repellents was continued by evaluating new and promising materials as systemics for body lice when fed to rabbits. Of the many compounds tested, butazolidin, tandearil, and GC-8266

were of particular interest in that they were highly effective in killing lice at dosages that did not obviously hurt the host rabbits or they remained effective over extended periods.

6. Mites, Ticks, and Fleas. Research to develop effective systemic insecticides which might be used to control or repel fleas was continued at Gainesville. Approximately 40 compounds were tested as systemic insecticides against oriental rat fleas on guinea pigs. Compounds which showed toxicity to fleas were generally effective for only a few hours after treatment. A further problem is the toxicity of some compounds to the guinea pigs at doses which show toxicity to the fleas. One compound at a dosage of 50 mg/kg caused complete mortality of fleas 5 hours after treatment and 85% mortality 3 days after treatment. Another compound at 10 mg/kg caused complete mortality 3 hours after treatment and became progressively less effective for the next seven days at which time no fleas were killed. Mirex fed to rats in their diet caused mortality of fleas feeding on the rats.

The search for effective residual insecticides to control fleas was continued through screening approximately 70 candidate compounds in standardized screening procedures or tests on treated soil. Approximately 1/4th of the compounds were equal to or better than the standard, malathion.

7. Bed bugs. At Gainesville, Florida, investigations were continued to find more effective insecticides for control of bed bugs. In standardized laboratory tests 69 candidate materials were evaluated of which 19 were more effective than the standard, malathion.

Four laboratory colonies of bed bugs maintained for resistance studies showed slight to moderate levels of resistance to malathion. DDT- and dieldrin-treated papers used in standardized resistance tests did not lose insecticidal effectiveness after 45 months of storage.

C. Biological Control

1. Mosquitoes. At Lake Charles, La., research on pathogens of mosquitoes has been conducted. Field collections throughout the area have shown infections of microsporidia in thirteen species including the genera Culex, Aedes, Culiseta, Anopheles, and Orthopodomyia. Aedes grossbechi and Orthopodomyia signifera were new host records for a microsporidian. Spore sizes were determined and classification of the microsporidia studied. Transovarial transmission of microsporidian infections was studied in 12 species of mosquitoes and demonstrated in seven of the species.

The fungus Coelomomyces was found in field collections infecting larvae of Culex restuans, C. salinarius, Aedes vexans, A. sollicitans and Culiseta inornata. Culex salinarius, C. restuans and Aedes sollicitans represent new host records for Coelomomyces. Field infection levels varied from very low to over 50 percent.

A polyhedral virus was reported from larvae of Aedes vexans and Psorophora ferox. Both species were previously unreported as hosts of viruses. Infection levels in the field were very low. A very lethal bacteria was collected from larvae of six mosquito species.

At Gainesville, Fla., two species of Thelohania were found in A. quadrimaculatus, one infecting the adipose tissue and the other the oenocytes. One species was found infecting the oenocytes of A. crucians larvae.

D. Insect Sterility, Attractants, and Other New Approaches to Control

1. Mosquitoes. At Gainesville, Fla., studies were continued on factors affecting the attraction of mosquitoes to their hosts and factors affecting the protection time from mosquito bites afforded by repellents. A large olfactometer was developed to study these factors as well as to evaluate the efficacy of various attractant materials or factors.

Fifty men were significantly more attractive on the average than 50 women to Aedes aegypti (L.). The average protection times of the women treated with 5% deet were significantly longer than men. The few subjects having unusually high skin temperatures were more attractive than the few subjects with usually low temperatures. The few women producing high outputs of moisture from a fore arm were more attractive than the few women with low outputs. The reverse was shown by the men. Women with low moisture outputs had longer protection periods than women with high outputs and no correlation was shown by the men. There was little or no correlation between color of hair of the subjects and attractiveness or protection time. The attraction to mosquitoes of the 37 women with menstrual cycles followed the cyclic endometrical changes.

An investigation was initiated to test hypothesis concerning attraction of mosquitoes to humans, particularly to determine if female mosquitoes fly downwind as well as upwind in search of human hosts. Data obtained do not deny olfactory location of hosts by Aedes aegypti, but they show that there may be other means of host-location in addition to olfaction, since mosquitoes were attracted to human hosts downwind.

Tests were conducted to determine the response of female Aedes aegypti to chloroform and human emanations when various flagellar segments of their antennae were removed. Not until six distal segments were removed did the overall attractant response show a decline. After amputations of 8 to 10 distal segments the decline in response leveled off. Both agents apparently affected receptive organs of the antennae in a similar fashion.

The effort to develop effective space and systemic repellents was continued and slightly expanded. To date several materials show some space repellency to mosquitoes in that they prevent mosquitoes from penetrating 4-mesh screening. Materials exhibiting some systemic repellency were found and further tests will be made on these materials.

Studies were continued at Gainesville, Fla., to evaluate materials as chemosterilants for mosquitoes and to evaluate the sterility principle of mosquito control. Tests with hempa as a chemosterilant indicated it would have little value as a residual sterilant for mosquitoes. Further selection and studies were conducted with the apholate-resistant colony of Aedes aegypti to clearly define the degree of resistance. This colony is at least 10 times as resistant to the sterilizing effects of apholate as the unselected, parent colony. Selections to increase resistance will be continued.

A sterile male release study was made of Anopheles quadrimaculatus in a semi-isolated area. This breeding site was made more favorable by increasing the number of breeding sites and introducing additional wild stock of this mosquito to populate the area. When sterile males of wild stock were released, sterility of the natural population increased from a very low degree up to 42%. When the releases were changed to sterile colony males, the sterility in the natural population decreased confirming earlier work showing behavior differences between colony and wild strains in seeking out wild females. Apparently a sufficient number of males was not released to reduce the population levels of A. quadrimaculatus.

Studies were continued on the evaluation of chemosterilants for mosquitoes at Corvallis, Oreg. The chemosterilant, hempa, was not highly effective in sterilizing Culex tarsalis larvae in that rates as high as 200 ppm were required. As a residual treatment in glass jars, 10 milligrams of hempa per square foot sterilized adult males completely, but adult females only partially. In wind tunnel tests against adults, a concentration of 10% caused high sterility, whereas a 5% spray caused only partial sterility and none was caused at 1% or lower. Males were generally more susceptible than the females.

At Corvallis, Oreg., studies were continued on sex and ovipositional attractants for mosquitoes. Preliminary tests indicated the presence of a sex attractant in Culex quinquefasciatus, but further tests did not confirm its presence, nor the presence of a sex attractant in Culex tarsalis. Many mosquitoes are known to choose specific types of water for oviposition. Studies have shown that odors from grass infusions and log pond waters collected in distilled water were attractive to gravid females of Culex quinquefasciatus. These odors were not attractive to females of C. tarsalis. However, log pond water itself was more attractive to this species than either distilled water or distilled water plus log pond odors. Distilled water saturated with methane was also attractive to gravid females of C. quinquefasciatus but not to those of C. tarsalis. Gravid females of both species were more attracted to distilled water treated with 25 ppm of furfural than to water treated with 5 or 50 ppm.

2. House Flies. At Gainesville, Fla., research was continued on the development of chemosterilants and the sterility principle of control for house flies. Several hundred new candidate compounds were evaluated in

primary screening and secondary development tests and many were found to exhibit sterilizing efficacy against both males and females. Particular attention was paid to evaluating two compounds, hempa and hemel, as sterilants by several routes of administration. These two materials will sterilize both sexes of the house fly.

Two series of field tests were conducted at poultry farms in Florida to evaluate the effectiveness of two chemosterilants, hempa and apholate, for the control of house flies. At the poultry farm treated with hempa, house flies were reduced in abundance from 71 per grid to 0 within eight weeks. Grid counts remained at zero for the remainder of the test period. At the poultry house treated with apholate, population levels decreased from 200 per grid to less than 10 within six weeks and remained constant at a low level throughout the remainder of the test.

Experiments on the Island of Grand Turk were continued. Application of chemosterilant bait in local privies had reduced population levels drastically to a good level of control but eradication was not achieved. Considerable biological research was undertaken which developed information on population levels before and after the use of chemosterilant baits, rate of increase of the population, migration and mating behavior. Techniques were developed to produce and sterilize (with gamma radiation) 2 million house flies per week and ship them to Grand Turk for release in an attempt to eradicate the house fly population on Grand Turk. Releases are in progress and results not yet known.

Ninety chemicals were screened as chemosterilants against adult house flies. Six compounds were toxic and 10 reduced the fertility to some extent in fly food or sugar. Thirty-two compounds, previously shown to sterilize house flies, were also tested again at higher or lower concentrations.

Tests were conducted with 21 compounds to determine their effectiveness as male house fly sterilants. Of the 14 chemicals, Olin 53330 (ENT-50838), Squibb Olin 53331 (ENT-50839), Squibb Olin 53356 (ENT-50840), and Squibb Olin 53263 (ENT-50842) sterilized at a concentration of 0.05% in the sugar diet. Sankyo Company RES-101 (ENT-50845) at this dosage in sguar induced sterility in the first two eggings.

A sterility test was conducted to determine if treatments on Grand Turk Island have induced genetic damage to the house fly population. Regular laboratory flies, an F_1 colony from a poultry farm near Gainesville, Fla., and an F_2 colony of flies collected from Grand Turk were used in the test. Results indicate that metepa produced genetic injury to the existing house fly population of Grand Turk. Sterility was still 44.8% with the Grand Turk flies reared for two generations in the laboratory away from metepa selection, compared to 9.0% with the laboratory flies and 7.6% with the field strain. The regular females laid 50% more eggs per female than Grand Turk females. Only 7 of 91 Grand Turk females laid over 100 eggs, as compared to 67 of 93 regular females. The percentage of eggs that hatched

was almost identical with all of the strains but pupation of the Grand Turk larvae was about 50% less than the other two strains.

Tests to determine if flies from Grand Turk Island, which have been under metepa pressure for the past 2 years, could regain their fertility when exposure to this chemosterilant was discontinued. Fertility tests have indicated that the metepa treatment causes approximately 80% sterility in the natural population. A field-collected strain of Grand Turk flies was reared at the laboratory for 9 generations. At the P, F_5, and F_8 generations, 1,000 eggs were collected on oviposition medium and their sterility evaluated following the procedure used in the preliminary screening tests. Fertility in Grand Turk flies increased rapidly from a low of 13.8% at the parent generation to 80.1% by the F_8 generation.

As a release program is now being considered on the island of Grand Turk, investigations were made to determine which of three strains of male house flies was most receptive by virgin females from Grand Turk. Males from the regular, Salt Cay (obtained from a small island about 8 miles southwest of Grand Turk), and Grand Turk strains were isolated as they emerged from the pupal stage and 5 days later were individually introduced into a cage containing 15 5-day-old virgin females from the Grand Turk colony. The Grand Turk females mated most readily with the Grand Turk males (61.3% mated). The Salt Cay males inseminated 41.2% of the females, and the regular males only 38.9%.

Investigations were continued on determination of the actual time house flies remained in copula after initial seizure. The results are given in the table below.

Colony and generation	No. of females tested	Mating period (min.) Longest	Shortest	Average
Regular, unknown	100	90	22	59.3
Poultry farm, F_1	74	110	30	47.6
Grand Turk, F_2	100	63	16	44.4

Field flies appeared to spend less time in copula than laboratory-reared flies.

Basic studies were continued on the cytological effects of chemosterilants on house fly reproductive systems and previous sectioning, fixing, and staining techniques have been used to study several new chemosterilants.

The olfactometers designed by Gouck and Schreck have been used to initiate a search for more effective house fly attractants. Nineteen compounds were tested as house fly attractants, using Edamin as a standard. Beef protein concentrate was attractive to females but not to males. The other materials were not as effective as Edamin.

At Corvallis, Oreg., research was conducted on chemosterilants and attractants for the house fly and little house fly. With the little house fly, hempa caused sterility as a residual deposit on glass at 50 mg/ft^2. However the sterilizing dose caused some mortality. Higher doses were highly toxic to adult flies; lower doses did not sterilize. When fed orally, hempa was toxic at 0.25% and lethal at 1.0%. Higher, but incomplete sterility was caused at concentrations as low as 0.01%. Topical treatments of hempa and hemel sterilized males without causing mortality, but not females. In general, treatments causing a high degree of sterility did not affect mating competitiveness. Four known antioxidants exhibited little effect on egg production or egg hatch. Gamma radiation greater than 1000 r of pupae prevented oviposition by emerging adults.

Research was continued on the sex pheromone in house flies. The presence of a low titre of pheromone in extracts of pupae and young females was shown and confirmed. Higher activity of the pheromone was demonstrated in 3-day-old flies. Presence of the pheromone was shown in females from strains of different origin. Males of different strains reacted to extracts from females of different strains, though differences were apparent.

3. Cockroaches. In Florida, research was conducted to develop effective cockroach repellents. Effectiveness is based on length of time before roaches enter treated cartons containing food and water. Phillips 55 was effective in that no more than 35% of test populations were observed inside treated cartons for at least 35 days and less than 50% for the following 2 weeks. Deet was effective for at least 3 days; pyrethrins was ineffective after the first day. The fencholic acid standard lasted a minimum of 7 days. Basic studies of the effects of chemosterilants on embryology and reproductive capacity of German cockroaches were conducted. A high degree of sterility was caused by administration of chemosterilants; however, the treatments caused some toxicity to the cockroaches.

4. Repellents--Mites, Ticks, and Fleas. Studies were continued at Gainesville, Fla., to find new repellents and acaricides for ticks and mites. Of 11 compounds tested for repellency to nymphal lone star ticks, 2 were highly effective. 4-(decenoyl) morpholine gave 95% protection for 96 days and 80% protection for 170 days. With chigger mites, screening tests were completed with 11 compounds. 2-methoxynaphthalene was effective after two washes but not after the third.

5. Tsetse Flies. Studies continued in Rhodesia on the feasibility of the sterile-male technique to control tsetse flies. Three of 4 chemosterilants were effective against 2 species of Glossina by one or more of 4 methods of administration. Field studies elucidated migration, behavior, and mating habits in nature and various types of cages, but rearing of flies in semi-natural or cage environments has not been accomplished.

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AREA NO. 18. BEES AND OTHER POLLINATING INSECTS

Problem. Bees contribute to the production of crops occupying at least 5 million acres. Their pollinating activities perform a necessary service in the production of fruit crops, such as, deciduous fruits, berries, cucumbers, melons, and also for vegetable and legume seed crops which are required for the planting of many hundreds of thousands of acres. This pollinating service is performed incidental to foraging for foods for their own use (nectar and pollen). Of all the insect pollinators honey bees are the most important.

Beekeeping is practiced in all 50 states. This distributes pollinators throughout all the cultivated areas. It makes available a manageable supply of pollinators for use where and when they are required. A self-supporting pollination service for agricultural crops is provided through the production and sale of honey and beeswax. To maintain an adequate level of pollinators, however, beekeeping must be kept in a profitable condition.

A problem of major significance is the increasing use of pesticides, many of which are hazardous to bees or destroy important pollen and nectar sources. There is need for more knowledge of the management of bee colonies; breeding of improved hybrid bees; physiology and behavior of queens, drones, and workers; and the various diseases and pests of the honey bee and means for their control. There is also need to study the many facets of the complex pollination problem to integrate effectively populations of honey bees and other pollinating insects with crop needs and practices. More knowledge should be obtained about wild insect pollinators and their management. It is also essential to study the effects of farm practices, such as the use of different pesticides, changes in crops, soil management, and harvesting, on the economy of the beekeeping industry and the survival of pollinating insects, and to develop procedures to minimize losses from such practices. Information is needed on nectar and pollen plants for use in conservation program efforts to provide bee forage areas in wastelands, watersheds, and roadsides. The nutrition of bees and the nutritive value of different pollens to bees require intensive investigation together with basic nutrition studies for development of pollen substitutes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program involving apiculturists, geneticists, microbiologists, physiologists, and entomologists engaged in basic studies and in research concerned with the application of known principles to the solution of crop pollination problems for the farmer and problems that affect the beekeeper. Bee breeding investigations at Baton Rouge, La., are cooperative with the State Experiment Stations of Louisiana, Ohio,

California, and Wisconsin, and the Ontario Agricultural College, Guelph, Ontario, Canada. Bee management investigations at Madison, Wis., are cooperative with the Wisconsin and Arizona Experiment Stations, the Department of Apiculture at Ontario Agricultural College, Canada, the Abbott and Pfizer Laboratories, and the Agricultural Engineering Research Division. Investigations on bee diseases are carried on at Beltsville, Md., and Laramie, Wyo., in cooperation with the Louisiana, Wisconsin, and Wyoming Experiment Stations. Honey bee pollination investigations at Tucson, Ariz., are carried on in cooperation with the Experiment Stations of Arizona, California, Louisiana, Utah, and Wisconsin, and the Agricultural Engineering Research and Plant Pest Control Divisions of ARS. Wild bee pollination investigations at Logan, Utah, are conducted in cooperation with the Experiment Stations of Arizona, Utah, Louisiana, Wyoming, Idaho, Oregon, Washington, the Crops and Agricultural Engineering Research Divisions, ARS, and private beekeepers and farmers.

The Federal scientific effort devoted to apiculture research totals 22.0 professional man-years. Of this number 3.5 is devoted to biology and breeding for improvement of the honey bee; 2.0 to management for improvement in productivity of honey bees; 6.5 to etiology of bee diseases and development of control methods for diseases and pests; 5.0 to behavior and utilization of honey bees in the pollination of agricultural and other economic crops; 2.0 to biology and utilization of insects other than honey bees in the pollination of agricultural crops; 1.0 to effect of pesticides, and farm practices on honey bees and other pollinating insects; and 2.0 to program leadership.

In addition Federal support of research is provided under a grant to Ohio State University for 0.6 man-year for bee disease research, specifically Nosema.

Apiculture research conducted under P. L. 480 grants total 13.25 man-years. Bee breeding research is being conducted at the Central Apicultural College, Warsaw, Poland (2.5 man-years) and at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Sao Paulo, Brazil (3.0 man-years). Bee disease research is underway with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (3.0 man-years) and with the Instituto Nazionale di Apicoltura, Bologna, Italy (0.75 man-year). Wild bee pollination research is being conducted with the Faculty of Agriculture, Department of Agricultural Zoology, University of Cairo, Egypt (1.5 man-years) and with the Government Agriculture College and Research Institute, Ludhiana, Punjab, India (2.5 man-years).

PROGRAM OF THE STATE EXPERIMENT STATIONS

The State stations have a good research program on bees and insect pollination. The studies on pollination cover honey bees, alkali bees, leaf cutter bees, bumble bees and other insect pollinators. The behavior, life history, morphology, taxonomy and ecology of pollinating insects, pollination of alfalfa and other legumes and fruits and vegetables as well as other plants, the attractiveness of the blooms to the bees, the effectiveness of different pollinators in terms of seed or fruit yield, and the more important pollinating insects in different areas and factors responsible for their effectiveness are being studied. Methods of protecting and increasing species of pollinators are being developed. Studies are being made to determine the effect of pesticide chemicals on pollinating insects. The nest, characteristics of nest sites and nest parasites of pollinating insects are being described. The biotic and abiotic factors affecting the establishment, maintenance of wild bee population and the climatic and other factors affecting bees visiting crops to pollinate them are being studied. The most economical numbers of colonies of honey bees per acre for honey production and the saturation point with reference to pollination for seed production are being determined. Management procedures for encouraging wild bees as pollinators are being developed. Wild and cultivated cucurbits are surveyed to determine the pollinating insects which visit them and the relative effectiveness of these insects as pollinators of these plants. The role of chemicals found in bee secretions as well as sounds which are used in normal communicative media in honey bee colonies and the use of colonies of honey bees for pollination including the effect of location, colony strength and training of bees on pollination are under study.

Bee resistance to disease and genetics are elucidated through physiological, ecological, behavioristic and genetic studies of the general characteristics in the honey bee including resistance and susceptibility to American foulbrood. Control of American and European foulbrood, and Nosema, by drugs and antibiotics including methods of feeding colonies, are being studied, as, is favorable apiary management to control or to retard the development of disease.

The nutrition of the honey bee is being studied. The amino acid requirements are being determined in an effort to develop a pollen supplement or substitute. Nutritional factors responsible for the differentiation and maintenance of castes of honey bees are being investigated.

Methods of queen rearing of honey bees, and the study of natural and artificial mating with the purpose of improving artificial insemination are being studied.

There are 21.7 professional man-years devoted to research on bees and pollination at the State stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

A. Biology and Breeding for Improvement of the Honey Bee

1. Alfalfa Pollen Collecting Strain. Honey bee colonies collecting a high percentage of alfalfa pollen and others collecting a low percentage have been selected at Logan, Utah, and progeny reared at Baton Rouge, La. Further testing has established that this character is heritable. This important find indicates that strains can be selected for pollination of specific crops or for other characteristics. Maintaining high viability of brood in these lines is difficult, an unavoidable problem associated with inbreeding within a line.

2. Circadian Rhythm in Drones. To measure the effect of a change in sun time of approximately 5 hours in the circadian flight rhythm of drones a small colony of bees was transferred by air from Louisiana to Hawaii and return. Drones and worker bees were confined in transit for approximately 16 hours during each trip with the drones missing one daily flight. The drones compensated for the change by three hours on their first flight and over-compensated by about 15 minutes on their second flight period 24 hours later. The mechanism for the over-compensation is not understood.

3. Flight Room Studies. At Baton Rouge, La., studies of a small colony of bees in a flight room indicated that control of the population can be affected by small but continuous feedings of sirup and pollen. Nosema disease can be controlled by feeding Fumidil B. Cessation of feeding the drug however, resulted in an increase of the disease.

4. Hybrids From Imported Stock. Some of the inbred lines of bees formerly maintained at the Baton Rouge laboratory have been discarded and new lines have been established. In certain specific hybrid combinations the Carniolan line has appeared superior to the Caucasian line. The Greek and Anatolian lines have shown superiority in some crosses with inbred Italian lines.

5. Bee Room. At Baton Rouge, La., a bee room to accommodate eight colonies of bees has been built. Inside temperature is maintained with a heat pump. The bees are permitted outside flight through plastic tunnels. Caged queens were successfully wintered on these colonies and early queen rearing has also shown considerable promise.

6. Controlling Nosema in Queen Rearing Colonies. At Baton Rouge, tests were made of methods of controlling Nosema infections in queen rearing colonies. Fumigation of equipment with acetic acid was ineffective. Feeding Fumidil B in sugar sirup apparently reduced the Nosema infection to zero, but after feeding was discontinued the infection reappeared.

Bee breeding research under P. L. 480 funds at the Central Apicultural College, Warsaw, Poland, (E21-ENT-7) has demonstrated that queen bees do produce diploid drone eggs. It is now proposed to carry these diploid drones to maturity and investigate their anatomy, histology, and physiology together with quality and quantity of semen produced.

Research on bee breeding is also being conducted at the Faculdade de Filosofia, Ciencias e Letras de Rio Claro, Rio Claro, Sao Paulo, Brazil (S3-ENT-1). Of particular interest is the observation in the Trigona bees that an unfed queen produces a drop of glandular food which is eaten by the workers, after they feed and attend her. This would appear to be related to "queen substance" of queen honey bees. It would be highly interesting to have such a droplet analyzed for the basic chemicals in "queen substance." A manuscript on morphology of Melipona is to be published also.

B. Management for Improvement in Productivity of Honey Bees

1. Honey Removal. High-volume low-pressure air blast was shown to be highly effective in removing bees from honey supers at Madison, Wis. Supers were removed in less than a minute with fewer bees left in them than by any other method.

2. Queen Storage. At Madison, caged queens can be overwintered in specially prepared queenless colonies with large populations and treated with fumagillin to control Nosema. Each queen is provided with queen candy containing 2% royal jelly.

3. Carbaryl vs. Bees. At Madison, a late August application of carbaryl on canning sweet corn without notification by the canning company led to weakening and eventual loss of over 50% of the laboratory's valuable bee breeding stock.

4. Peritrophic Membrane Development. Differences in the rate and extent of development of the larval peritrophic membrane were demonstrated between 8 inbred lines. The relation of this difference to disease control and other management factors will be tested in the future at Madison.

5. Bee Repellents. At Madison, the exposure of honey to the repellents propionic anhydride, 25% acetic acid and benzaldehyde evaluated by 27 panelists indicate that there is no significant pick up of flavor at exposures expected within good management practice.

The effectiveness of the repellents propionic anhydride, dilute acetic acid, benzaldehyde, and butyric anhydride was compared on several days under varying temperatures and conditions. The repellents propionic anhydride and butyric anhydride are most effective at temperatures around 80° to 90° F. Dilute acetic acid plus smoke and benzaldehyde are most effective at

temperatures in the cooler range around 60° to 75° F.

6. Honey Production by Hybrids. At Madison, the (6ZX)(MDY) hybrid produced an average of 317 pounds of honey per colony, 35 pounds more than the average of all other hybrids tested against it. Package colonies inoculated with Nosema when installed in hives at Madison were treated with Fumadil B in sugar sirup, in powdered sugar and in queen candy. Control of Nosema disease was in proportion to the amount of medication consumed.

7. Food Consumption vs. Temperature. At Madison in a chamber having controlled temperature and humidity, caged bees increased their food consumption as the temperature dropped from 80° to 45° F. Water consumption increased as humidity decreased or as temperature increased.

To determine the feasibility of a "large, economy size", a 15.5 pound package of bees instead of the usual 3-pound size was given 5 caged queens, placed in a 71° F laboratory at Madison and observed for 2 days. The cluster temperature rose to 104° F, which caused death of all the queens and half the bees. Continued studies under lower temperature conditions will be made.

8. Pollen Collection. At Logan, Utah, bees in a controlled flight room collected reconstituted pollen, sirup, and water most actively at 10% RH. When the relative humidity was raised, pollen collection decreased and finally stopped at 80% RH. As humidity rose broodrearing also decreased. This seemed to be associated with difficulty in pollen collection at higher humidities. For maximum performance of bees in a flight room, dehumidifying equipment should be used.

9. Bumble Bee Honey. The honey removed from four species of Wisconsin bumble bees was of two types - "thick" and "thin". With only one exception (a nest of B. americanorum), "thick" bumble bee honey was found to be thinner than its honey bee equivalent, ranging around 79.5% sugars and 51.0% in "thin" honey, as compared with a range of 82.9 to 84.3% in honey bee honey over the same period.

C. Etiology of Bee Diseases and Development of Control Methods for Diseases and Pests

1. Disappearing Disease. No evidence of disease or toxic material was found in bees, comb, or pollen from colonies in the area of Louisiana where so-called disappearing disease occurs. Electron microphotographs of particular material found in some bee samples taken from colonies affected with disappearing disease showed two viruses of about 30 and 60 microns.

2. Nosema. Twenty days after installation of 20 packages of Louisiana bees in Beltsville, Md., the percentage of Nosema had risen from 15% to 95% and 5 colonies had become queenless. The 15 queenright colonies showed a high percent of infection until late July.

At Baton Rouge, La., samples of worker bees, when examined for Nosema apis spores, showed that infection was heavy in some apiaries and light in others. The peak of infection was in February, March, and April, with a rapid decline in May and June. Some queen cell starting colonies continued to show Nosema infection of 30 to 40 percent after most other colonies were apparently free of the disease. No increase in the incidence of the disease was observed in July and August as has been noted in previous years. In a commercial apiary the feeding of Fumidil B did not reduce the amount of Nosema in the colonies. However, in queen rearing colonies the medicant reduced the amount of Nosema. Treatment of baby nuclei combs with glacial acetic acid prior to the establishment of bees on these combs showed promising results in the reduction of Nosema in these queen rearing units.

At Laramie, Wyo., caged honey bees inoculated with Nosema apis spores and kept at high relative humidity (about 77%) developed Nosema disease in a larger percentage of individuals, and showed a greater infection per individual bee, than their sisters kept at low humidity (about 32%) at the same temperature (about 94° F). However, control cages for these tests were inadequate, probably because some bees failed to consume or regurgitated the sugar sirup inoculum. Protecting honey bee colonies during the winter with a black plastic packing around the hives did not affect the incidence of Nosema disease in early spring.

At Madison, Wis., the microsporidian Nosema apis once considered specific to Apis mellifera L. has now been demonstrated to be infectious in Bombus fervidus Fabr. Spores of N. apis, when ingested by B. fervidus, produced infection after 19 to 21 days. The parasite sporulates and great numbers of spores are found in the ventriculus. When these spores were fed to Nosema free A. mellifera, heavy infections developed within 7 days. The spores were identified as N. apis.

Spores of N. apis subjected to infrared heating at 100° F or 130° F do not lose their viability. The only changes observed were in the morphology of first-generation spores.

3. European Foulbrood. Bees were used extensively in southern New Jersey for pollination of blueberries and cranberries. When the colonies are moved from blueberries to cranberries European foulbrood appears and becomes a problem. A cooperative project between the Beltsville, Md., laboratory and Rutgers University was initiated to determine whether nutrition is a contributing factor to the increase of European foulbrood. Colonies were checked for disease going into cranberries early in June and again after coming out of cranberries. The control colonies showed the expected rise in disease. Colonies receiving sugar candy or sugar candy plus added vitamins showed 80 to 100% reduction of disease. Colonies receiving protein substitute cakes showed heavy development of the disease. However, these

cakes were poorly accepted by the bees. Colonies receiving spores of Bacillus alvei each showed no significant increase or decrease of disease. Colonies receiving 50 ml each of Alvein - the extracted antibiotic of B. alvei - showed no significant increase or decrease in disease.

4. Wax Moth. At Beltsville, Md., ethylene oxide tests with the greater wax moth Galleria mellonella using a concentration of 32.5 mg of ETO/liter gave the following results: 100% mortality with adults at 5 minutes exposure, pupae at 90 minutes exposure, larvae at 60 minutes exposure and eggs at 30 minutes exposure.

At Tucson, Ariz., preliminary tests to determine the dosage of ethylene oxide required to kill all stages of the wax moth were conducted. At temperatures above 90° F dosages as low as 5-10 mgs per liter for 24 hours seemed to be effective. At lower temperatures the same dosages did not kill all mature larvae or pupae. At current prices, dosage requirements of 10 or more mgs per liter would cost three times as much as methyl bromide to fumigate combs.

5. American Foulbrood. At Laramie, Wyo., repeated subculture of Bacillus larvae, the cause of American foulbrood, on Foster's artificial culture medium, decreased its virulence to 18 to 24 hour old honey bee larvae about 30%, and to 24 to 48 hour old larvae about 20%, compared to the virulence of spores recently isolated from the same American foulbrood scale collection.

Three gorgings of bees, in American foulbrood diseased colonies, with sugar sirup containing 1 gram tylosin lactate per gallon of sirup at approximately weekly intervals eliminated light infections. A strain of Bacillus larvae resistant to 10 micrograms/ml terramycin was isolated, but it failed to sporulate in artificial culture. Pieces of brood combs were completely sterilized by fumigation with ethylene oxide gas in a "Cryotherm" chamber at room temperature without vacuum, at a dosage of 0.372 gm/liter for 24 hours. However, honey absorbed the gas and was then toxic to honey bees. Methods of dissipating the gas from honey are being studied.

6. Sac Brood. Artificial inoculation of individual brood cells containing sister honey bee larvae of various known ages with filtrates of diseased sac brood larvae, two different age groups being inoculated at the same time with the same inoculum, confirmed our previous finding that larvae 1 to 3 days old are highly susceptible, those 3 to 4 days old are moderately, and those 4 to 5 days old are slightly susceptible to this disease at Laramie, Wyo.

7. Amoeba Disease. At Laramie, Wyo., attempts to infect caged honey bees with Malpighamoeba mellificae by feeding the cysts in 20% sugar sirup, either with or without 4% pollen, failed to produce amoeba disease, either by mass feeding from feeder vials for 24 hours, or by feeding individual bees

from a pipette to insure that they ingested the inoculum. The cages were kept at about 78° F and at a comparatively high relative humidity (about 75%). Possibly the cysts, which had been refrigerated several months, had lost their viability, or conditions were unfavorable for their development.

8. Mites. Acarapis dorsalis mites were found externally on the backs and A. vagans mites on the wings on about 6% of the honey bees from California. No neck mites, A. externus, were seen. The same two external mite species were found on a small percentage of honey bees from one of our apiaries at Laramie, Wyo., in March 1965.

In work on Acarine disease control under P. L. 480 project E15-ENT-1 in Italy, 30 products (vapors or orally applied) were tested which proved to be either too toxic to bees or ineffective against the mites. It was found that the mites normally invade the air sacs of the bees head. In the winter cluster, old bees as well as young ones may become infested. No method of artificial culturing has been successful. Mites of the Acarapis genus were found on bodies of Vespula but the species have not been determined.

Research conducted under P. L. 480 project A7-ENT-10 in India indicates that mites generally infest young bees up to 4 days old. They enter trachea as mated females and lay eggs which hatch in 1 - 2 days. Blackened trachea is noticeable in about 4 days. In dead bees mites may live 24 to 72 hours. Folbex fumigation at weekly intervals gave good control. In the continued survey, mite infested colonies of Apis indica bees were found in the states of Jammu and Kashmir. No Acarapis infestations have been found in Apis dorsata or Apis florea colonies examined in 7 states of India.

D. Behavior and Utilization of Honey Bees in the Pollination of Agricultural and Other Economic Crops

1. Safflower. In commercial safflower fields in Arizona 5 times as many bees were near the edges of the fields as near the center. The relative number of bees present was related to nearness of commercial apiaries. Wild bees in all cases made only a small contribution to the pollinator population.

Honey bee activity on safflower almost ceases after noon. The bees show a strong tendency to work along, rather than across rows. Both nectar and pollen collectors showed preference for yellow flowers over white but remained faithful to the color they visited. A flower-per-bee ratio appears to be a promising method of comparing pollinator populations in different fields.

2. Alfalfa. In Tucson, Ariz., before landmarks for bee orientation were erected in an alfalfa field only 8% of marked bees were seen again compared to 20% after landmarks were erected. In another field the figures were 13.5% before and 20% after landmark erection.

Of 80 materials screened for honey bee attractance or repellence only one stimulated strong attractant response and 6 were highly repellent.

3. White Clover. At Baton Rouge, La., studies on seed production in white clover, the trace elements boron, copper, zinc and molybdenum were added to experimental plots. While no significant differences in honey bee activity between plots was measured and seed yields were smaller than desirable from a commercial standpoint, significant differences were noted in the various treatments. The addition of boron at the rate of 2 pounds per acre resulted in a significant increase in white clover seed production. The application of boron resulted in more fertile florets per head and therefore more seed per plot. The application of copper, zinc and molybdenum were not significant in these tests.

4. Pollen Diets. At Tucson, pollen and pollen substitutes were fed to newly-emerged bees for 7 days then the nitrogenous products determined in the fecal material. Uric acid excretion varied inversely with diet adequacy.

Sterols were separated from a group of selected honey bee gathered pollens. The level of 24-methylene cholesterol reached 30% of the total sterols in black locust but was negligible in cottonwood and black cherry pollen.

E. Biology and Utilization of Insects Other Than Honey Bees in the Pollination of Agricultural Crops

1. Wild Bee Pollinators. Testing of materials for use in preparing leaf-cutter bee nests was continued at Logan, Utah. In general mortalities were lowest in straws. The best 5 materials were straws of the following diameters and treatment: 3/32" unwaxed and unembedded, 7/32" waxed and embedded, 6/32" unwaxed and unembedded, 7/32" waxed and unembedded, 6/32" waxed and unembedded.

Tribolium madens, a pest of leaf-cutter bee nests, was controlled with DDT-treated honey bee pollen pellets in a one-pellet layer on the nest-box floor where adult bees could not reach them.

The sex of Nomia prepupa was checked progressively during the nesting season. In mid-July about 25% of the prepupae were males, at the end of the nesting season the percentage had risen to about 50. During the latter part of the nesting period males were relatively scarce. At the end of the nesting period many of the females were almost spermless. This suggests that like honey bees Nomia have haploid males and diploid females but the females less efficiently maintain their sperm reservoir.

F. Effect of Pesticides, Insect Diseases, and Farm Practices on Honey Bees and Other Pollinating Insects

1. Monitoring. Two colonies of honey bees were placed on each of the 10 DIAP (Determination of the Impact of Agricultural Pesticides) monitoring plots in Arkansas and Mississippi in June 1964 and observed throughout the remainder of the period. Severe bee kills were observed following each application of pesticides to cotton. Although colony populations were reduced more than 50% in some areas no colonies were lost. Pesticides were found in dead bees and in pollen but no sample of honey contained pesticides in sufficient quantity to be considered unsafe for food at the levels of tolerances measured. The presence of pesticides in some pollens but not in others is of major importance. Likewise the presence of pesticides not known to have been applied in that area during the present season but applied in previous years, in some pollens, bees, and soils is of considerable interest. These determinations were made by Plant Pest Control Division chemists on materials (bees, pollen, and honey) collected by Baton Rouge, La., personnel.

2. Malathion Spray and Bees. The profits of commercial beekeeping may be greatly reduced by insecticide usage. Low volume malathion spray applied for grasshopper control in July near Buffalo, Wyoming, destroyed the field forces of 600 colonies in 15 apiaries. The full consequence of this application could not be determined before the following May. The colonies produced no honey crop nor did they store enough pollen and honey to sustain them (unaided) through the winter. As a substitute for honey stores all colonies were fed 35 pounds of sugar, but, more than half of them died before spring. Forty colonies were set aside for special study. To half of these 700 pounds of sugar was fed, only 8 survived the winter. The other 20 were doubled up in the fall and fed 350 pounds of sugar and these 10 survived the winter.

At Yakima, Wash., honey bees found near hives that had been in an area treated by aircraft with 9.74 ounces of actual technical malathion per acre were analyzed for the Plant Pest Control Division. Malathion residues were found in the dead bees in the amounts of 7.6 and 11.6 ppm. Live bees and pollen did not contain malathion residues above the limit of sensitivity of the method of analysis used (1.5 ppm).

In cooperation with the Plant Pest Control Division and the Washington State University bees and alfalfa were analyzed at Yakima, Wash. Six colonies of bees were placed in an alfalfa field in bloom, just before treatment of the field by airplane with a low volume spray of technical malathion at the rate of 10 ounces active material per acre. Live bees taken 29 hours after treatment from five of the colonies and from a check colony (2.25 miles from the treated field and displaying some malathion poisoning symptoms) contained residues ranging from less than 0.2 to 0.5 ppm of malathion. Dead bees

taken from the five colonies 36 hours after treatment contained from 3.2 to 5.7 ppm malathion, while the check colony dead bees showed 1.6 ppm. One check and the sixth colony were kept covered for 48 hours. At three days they contained 1.6 and 2.5 ppm malathion. At five days the bee loss had decreased significantly and at that time three of the six colonies showed no measurable malathion in dead bees. There was great variation between duplicate colonies.

3. Herbicides vs. Bees. Two experiments were run at Madison, Wis., to determine the toxicity of four widely used herbicides - Weed-out, Banvel-D, 2,4-D, and 2,4,5-T to honey bees. Experiment 1 used 30 grams of bees in contact with 1000 and 3500 parts per million solutions of the above herbicide on saturated masonite boards. Mortality records for a 28-day period showed 2,4-D at both levels and Weed-out at the higher dosage to be 25% to 50% more toxic than the other chemicals and the check.

The second experiment employed 30 grams of caged bees fed on sugar sirup containing 1000 and 3500 parts per million of the four herbicides. Mortality records for 3 weeks showed all but 2,4,5-T to be 25% to 50% more toxic than no treatment. The conclusion was reached that these herbicides used in quantities on flowering plants visited as forage for bees could result in slight loss of forage bees through contact and ingestion of the herbicides.

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AREA NO. 19. ANALYSIS, SYNTHESIS, FORMULATION, AND EVALUATION OF INSECT CONTROL CHEMICALS

Problem. Modern insecticides are a rapid and effective means of controlling injurious insects and their use has enabled the American farmer to produce an abundance of high quality crops and livestock. This extensive use, however, has been accompanied by increasing resistance of some insects to certain insecticides and by the possibility of leaving harmful residues on or in harvested crops, in meat, or in dairy and poultry products. There is therefore a need for the development of new types of chemicals, from natural sources and through synthesis, to which insects will not become resistant. These chemicals should be safe to handle and not leave harmful residues in products used for foods or feeds, or adversely affect wildlife, beneficial insects and other desirable organisms. More effective and safer formulations of chemicals should be developed for the control of different insect species under various environmental conditions. Such chemicals and formulations require initial testing in the laboratory and evaluation under field conditions before they can be recommended for practical use. It is essential that accurate, sensitive analytical methods be developed for the determination of the amounts of chemicals deposited and the rate of disappearance of their residues and breakdown products in treated crops, animals, or soils. Better attractants as well as lures for additional important insect pests are needed for use in traps and bait sprays for both insect detection and control. Research also is needed on repellents that would be useful in controlling insect attacks on crops, livestock, and man. Insect chemosterilants appear promising for use in insect control and their potentialities should be thoroughly explored.

USDA AND COOPERATIVE PROGRAM

The Department has a long-term program involving chemists, entomologists, and scientists of other specialized disciplines to discover and develop new and improved insect control chemicals and methods of applying them. Chemical research to discover, isolate, and identify products of natural origin which can be employed for insect control is carried on mainly at Beltsville, Md. Components of the cotton plant that serve as attractants, arrestants, feeding stimulants, essential nutrients, or otherwise affect the boll weevil are being investigated at State College, Miss., in cooperation with the Mississippi Agricultural Experiment Station. Some chemical investigation of the natural sex attractant of the female codling moth is in progress at Yakima, Wash. Grants have been made to the University of Michigan for research on the sex attractant of the tobacco budworm and to the University of Wisconsin for research on the tobacco hornworm. Chemical research on synthetic organic materials and formulations for insect control is carried on at Beltsville, Md.; Gainesville, Fla., and State College, Miss. A contract has been negotiated with the Midwest Research Institute in Kansas City, Mo., for the synthesis of compounds needed in the research on insect attractants and chemosterilants. Development of analytical methods for insecticide residues is carried on at Beltsville, Md.; Tifton, Ga.; Kerrville, Tex.; and Yakima, Wash.

There is cooperation with the State Experiment Stations in the respective regions of these laboratories. Cooperative work with the States on insecticide residues is conducted in connection with the following Regional Research Projects: NC-19, Fundamental Problems Associated with the Accumulation of Pesticidal Chemicals in Soils; NC-33, Pesticide Residues on or in Food, Feed, and Forage Crops; NE-36, Determination of Pesticide Residues on Raw Agricultural Commodities; NE-53, Transformation of Insecticides by Plants; S-22, Pesticide Residues on Plant and Animal Products and Soils; W-45, Pesticide Residues, Their Nature, Distribution, and Persistence in Plants, Animals, and Soils; and Interregional Project IR-4, Evaluation of Current Data and Needed Research to Obtain Clearance for Safe, Effective Chemicals for Minor Uses on Agricultural Products. Research on aerosols for insect control is conducted at Beltsville, Md. Biological evaluation of insecticides and other types of insect control chemicals is carried on at Beltsville, Md., and Brownsville, Tex. Research on methods for control of insects in aircraft is done at Beltsville, Md.

The Federal scientific effort devoted to research in this area totals 44.0 professional man-years. Of this number 9.8 are devoted to products of natural origin as sources of insect control materials; 19.2 to development of synthetic organic materials and formulations for insect control; 4.8 to methods of analyses for insecticide residues; 1.0 to aerosols for insect control; 7.0 to biological evaluation of insect control chemicals; 0.2 to methods for control of insects in aircraft; and 2.0 to program leadership.

In addition the Federal support of research under contracts and grants provides 3.2 man-years in this area. Of this total 2.4 is devoted to products of natural origin as sources of insect control materials and 0.8 to development of synthetic organic materials and formulations for insect control.

PROGRAM OF THE STATE EXPERIMENT STATIONS

The State stations have an extensive program on the analysis and determination of insecticide residues which includes some research on acaricide residues. Instrumental, chemical and biological determinations of insecticide residues are made. Residues of insecticide are determined in/on crops and animals and their products and in soils and water. Residues are determined periodically from the day the insecticide is applied through harvest and in some cases during processing or marketing. Studies are made of changes that occur in pesticides after they are applied to plants. These changes include both those on and in the plants. One station is studying the effect of the plant cuticular waxes on insecticides. The effects of insecticides in soils upon growth, flavor and yield of various crops and upon the chemical, physical and biological properties of soils are determined. Pesticide soil colloid interactions are being studied. The occurrence of pesticide residues in tissues of living animals is also being studied as well as in animal

products. Poultry and eggs are being analyzed to determine if any pesticide residues can be found in them.

Research is in progress to determine the total fate of insecticides applied to crops. This includes the amounts and nature of the products disappearing by volatility, metabolic changes, translocation and other ways.

Research is in progress on the drift of insecticides. This is a very serious problem especially in intensive agricultural areas where different crops are grown in adjoining fields. Pesticide residues in milk are being determined. On-the-farm practices which may contribute to the occurrence of insecticide residues in milk are being studied. Work is going forward on the evaluation of current data and needed research to obtain clearance for safe effective pesticide chemicals for minor uses on crops.

Insecticide residue analytical methods are being improved and adapted to various crops, products, soils and water. Clean up of samples and methods of sampling crops and animal products are under study to obtain more reliable results with the minimum amount of work. Cooperative research is in progress on the standardization of methods of insecticide residue analysis.

The State stations do extensive research in field evaluation and residue analyses of insecticides and acaricides for the control of insects and mites attacking particular crops and animals. However, this research is not covered in this section as it is reported under the specific crops discussed elsewhere in the report.

Some stations make laboratory evaluations of the potential effectiveness of chemicals as insecticides and acaracides. These are often tested against resistant strains of insects. One station tests about 800 to 1000 new compounds each year. These are received from more than 20 firms for evaluation of their insecticidal or acaracidal properties. Fungus products are tested for toxicity to arthropods.

Research is underway on insecticide formulations through the study of the physical and chemical properties of carriers and solvents used in preparation of insecticide concentrates. The effects on the stability, dispersion, application and coverage by toxicants and the leaching of insecticides from granular formulations are determined.

Contact, ingestion and fumigation effects of insecticides are determined. Interactions and compatability studies are made. Potential hazards from fire and explosion of fumigants are investigated.

A total of 83.0 professional man-years of research is devoted by the State stations to this area.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

A. Products of Natural Origin as Sources of Insect Control Materials

1. Insect Sex Attractants. Chemical investigations on the isolation and identification of the natural sex attractants of a number of important insect species being carried on at Beltsville, Md. The sex attractant produced by virgin females of the pink bollworm moth has been identified chemically and a 16-step synthesis of the attractant has been devised and is in progress to fully confirm the structure. Extracts of pink bollworm attractant are being prepared from the females and supplied regularly to the Plant Pest Control Division for survey purposes in the Southwest and for research on use of the attractant for control. Column chromatography of extracts from female banded cucumber beetles has yielded a highly active sex attractant fraction. Work on isolation of the attractant is under way. Progress also has been made in purifying an active fraction of southern armyworm sex attractant.

The attractancy of gyplure (the synthetic gypsy moth attractant discovered by USDA scientists) has been shown to be masked by the presence of as little as 5% of ricinoleyl alcohol. This alcohol is one of the starting materials in the production of gyplure and its presence in commercial lots of the attractant has caused difficulty. A purification process has been developed and used to prepare batches of pure gyplure for use in survey and in aerial drop tests and experiments on control of the gypsy moth by confusion of the males. Electrophysiological methods have been used to study the response of male gypsy moths to the natural sex attractant, to gyplure, and to related compounds (see section E.2).

A study was begun of the masking of activity in the American cockroach sex attractant by substances present in extracts of the females.

In cooperation with Canadian entomologists, extracts of sex attractant from female oriental fruit moths were prepared and fractionated at Beltsville.

At Beltsville investigation of a sex excitant secreted by female house flies was undertaken and extracts were prepared and fractionated. Crude extracts of sex attractants from several other insect species were prepared for entomological tests. Investigation of the imported fire ant's trail marking substance was continued.

At Yakima, Wash. extracts of sex attractant from female codling moths were prepared, fractionated, and purified to a considerable degree.

2. Materials of Plant Origin. At Beltsville, Md. extracts and fractions were prepared from several plant species for testing for insecticidal, insect attractant, or repellent properties. Samples of extracts also were sent to the National Institutes of Health for antitumor tests.

At State College, Miss., in cooperation with the Mississippi Agricultural Experiment Station, constituents of the cotton square(bud) were investigated in connection with studies on substances in the cotton plant that attract or stimulate feeding by the boll weevil. It was shown that at least 17 fatty acids were present in the lipids of field grown cotton squares, of which 57% were C_{18} unsaturated acids. In connection with this work a procedure was developed for separation of organic acids by thin-layer chromatography of their 2,4-dinitrophenylhydrazides. Terpene hydrocarbons from the squares of Deltapine Smoothleaf cotton were isolated and identified. Components of the square that stimulate feeding by the boll weevil were extracted with various solvents and fractionated by column chromatography. This study provided evidence of the presence of three components having diverse chemical characteristics, which elicited response from the weevil. Response was modified by factors such as pH, light, diet, and age and sex of insect.

As part of a study on nitrogen metabolism in the boll weevil, feces of this insect were examined for non-protein amino acids. Twenty amino acids were detected and estimated in an ethanolic extract. Acid hydrolysis increased this number to 23. Free and bound non-protein amino acids and ammonia accounted for 3.23% of total feces nitrogen.

Feeding tests were conducted to compare the utilization, consumption, and preference by larvae of grain insects for various plant foods. The fall armyworm, corn earworm, and tobacco budworm were included in the tests. Samples of lyophilized plants or dry seeds were ground, mixed with agar and chromic oxide and prepared as gels. The larvae were placed in vials with the gels. After a 48-hour feeding period the excreta were removed from each vial, composited, dried, and samples of the excreta, and of the diet dried in the same manner, were analyzed to determine the percent utilization by the method of McGinnis and Casting. Utilization of the plant materials ranged from 16 to 69%. Chromic oxide, agar, or cellulose powder were not utilized.

B. Development of Synthetic Organic Materials and Formulations for Insect Control.

1. Preparation of Synthetic Organic Compounds for Testing as Insect Chemosterilants. A quantity of the chemosterilant tepa labeled with radioisotope C^{14} was synthesized and utilized in studies of the metabolism of tepa in house flies and boll weevils. In an investigation at Beltsville, Md. on house flies a large proportion of the radioactivity was recovered in the excreta from the C^{14} tepa-treated flies but previous work had revealed the

absence of aziridinyl compounds in the excreta. A negligible amount of volatile metabolite was recovered from the treated flies. A study at State College, Miss., on male boll weevils indicated that topically applied tepa was almost immediately absorbed into the haemolymph. Distribution of injected and topically applied tepa in different body parts was determined. About half the original radioactivity was excreted in the feces or expired as CO_2. Over 85% of the residual radioactivity in the body of the boll weevils after 2 days was composed of low molecular weight constituents.

Male boll weevils were effectively sterilized by tepa by feeding 1500 ppm in the diet for 2 days or by injection of 3.5 μg. Lower levels provided transitory sterilization.

A quantitative colorimetric method of analysis for tepa residues on insects was developed based on colorimetric determination of the aziridinyl function. The method was used to determine the amount of tepa on Mexican fruit fly puparia dipped in 5% tepa solution and also the amount on the adult flies that emerged from the treated pupae. About 1.5 micrograms of tepa per fly was found on the newly emerged adults and more than 90% of this disappeared within 3 days.

A second method of analysis was developed for tepa on codling moths, comprising thin layer chromatography of an extract from the moths followed by a total phosphorus determination on the eluate of the spot from the chromatogram.

Two series of compounds related to tepa and hempa were synthesized for a study of structure-activity relationships. The first series consisted of bis-(1-aziridinyl)alkylaminophosphine oxides and the second series consisted of compounds intermediate in structure between tepa and hempa [$\underset{\underset{0}{\|}}{P}(-N\triangleleft)_3$.

$Me_2N\underset{\underset{0}{\|}}{P}(-N\triangleleft)$, $(Me_2N)_2\underset{\underset{0}{\|}}{P}-N\triangleleft$, and $(Me_2N)_3\underset{\underset{0}{\|}}{P}$].

In the first series the sterilizing activity on male house flies decreased with increasing length of the alkyl carbon chain, except that the isopropyl compound was more effective than the ethyl. In comparison to tepa the first compound in the series was found to be a more effective chemosterilant whereas the other members of the series were either equal or less active than tepa as house fly sterilants. In the second series progressive replacement of aziridinyl groups by dimethyl-amino groups led to a regular decrease in sterilizing effect.

Series of compounds of a number of other structural types were synthesized for evaluation of insect chemosterilant properties. These included various s-triazine derivatives, unsymmetrically substituted phosphoramides, aziridines fused to ring systems, heterocyclic amines and amine oxides, cyclic

ureas and thioureas, aryl imines, etc.

At Gainesville, Fla., an investigation of the mechanism of apholate resistance in Aedes aegypti mosquitoes was undertaken and is still in progress. In connection with the study of sterility in insects a gas-liquid chromatographic column was developed that permits sufficient resolution of hydrocarbons present in cockroach haemolymph for mass spectral studies.

2. Preparation of Synthetic Organic Compounds for Testing as Insecticides, Insect Attractants or Repellents, or Synergists. In the investigation at Beltsville of synthetic attractants for the Mediterranean fruit fly it was shown previously that the commercial grade of trimedlure, a powerful attractant for the fly, is a mixture of four geometrical isomers. The four isomers have been separated by preparative gas chromatography and the structure and configuration of their molecules has been determined. In all four of them the methyl group is trans to the ester group. The two isomers with the axial configuration of the chlorine atom are more attractive than their congeners with the equatorial chlorine. One isomer is essentially inactive.

The volatility of various insect attractants was investigated. n-Butyl diethylmalonate was found to inhibit the crystallization of trimedlure and it has the same volatility as the attractant. It may prove useful in formulating Mediterranean fruit fly lures for use in cool areas.

The activity of the crystalline sludge remaining from the production of the winter grade of trimedlure is being investigated to determine its possible usefulness as an attractant.

A number of analogs of siglure as well as other types of compounds have been synthesized for evaluation as Mediterranean fruit fly attractants.

A study is being made of synergists for carbamate insecticides. A large number of compounds are being evaluated for this purpose, with particular emphasis on those containing a methylenedioxyphenyl group.

Four compounds that were effective as mosquito space repellents (repellent at some distance) in laboratory tests were synthesized in larger quantities for field testing. A number of analogs of these compounds also were synthesized for testing.

Improvements were made in the catalyst and techniques used in carbon skeleton chromatography for determination of the structure of organic molecules. These improvements have considerably extended the range of carbon chain lengths and types of compounds that this method can be applied to.

At Gainesville, Florida, the insect repellent 2-ethyl-1,3-hexanediol was separated into two constituents which are threo and erythro diastereoisomers. In tests against Aedes aegypti mosquitoes the isomers showed equal repellent activity.

3. Formulations. In research at Beltsville, Md., on improved formulations, experimental low-volume applications of undiluted technical malathion sprays deposited about twice as much insecticide on spinach and bean plants as did conventional dilute emulsion sprays at the same dosage of active ingredient and the deposits persisted more than twice as long. This more efficient deposition would permit less frequent applications. Low volume applications of other insecticides are being investigated.

At the request of the Bureau of Mines, U. S. Department of Interior, samples of fly ash are being studied as prospective carriers for insecticides. The physical properties of both granular and dust form fly ash seem suitable for applications in which the intense black color would not be objectionable. This material is available in large quantities as a waste product.

Spheroidal granules of attapulgite have been compared with the corresponding grade of conventional, irregularly shaped granules. The spheroidal form proved to be more absorptive, bulkier, and faster flowing than the conventional form.

In response to a request from the Pan American Sanitary Bureau for assistance, an investigation was made of an acute problem of poor suspensibility of DDT powder encountered in Mexico in connection with the malaria control program. It was found that the poor behavior of the powder was due to inadequacy of the surfactant ingredients. There also was evidence of faulty storage or testing in the field.

A study in cooperation with Animal Disease Eradication Division on 25% lindane water-dispersible powder for sheep dip indicated that for this purpose it is feasible and necessary to set a higher standard of dispersibility than has been adopted for any other water-dispersible insecticide powder.

Samples of 1% lindane dust stored by the Armed Forces for 14 years were tested and found to conform to specifications as regards physical quality, although there was noticeable rusting of cans. Analyses showed about 0.7 to 0.85% lindane present.

Studies were made in cooperation with Crops Research Division on the phytotoxicity to greenhouse plants of a number of solvents with dichlorvos. No injury was observed with methylene chloride, very little with Deobase-methylene chloride, more with xylene, and most with heavy aromatic naphtha. A group of synthetic isoparaffinic oils of unusually high purity, called

Isopars®, were tested on several varieties of chrysanthemums. The Isopars are essentially free of acids, sulfur, carbonyls, and chlorides and contain less than 0.5% by weight of aromatics. Isopar E (boiling range 240-286°F., unsulfonated residue 98%) and Isopar M (boiling range 403-480°F., unsulfonatable residue 98%) at 1 or 5 gals. per acre caused little or no injury to Pink Champagne, Yellow Shoesmith, Yellow Shasta, Nightingale, Mefo, Princess Anne, or Indiana Bronze chrysanthemums. When dichlorvos was added to these oils there was injury to Yellow Shasta and Nightingale.

At Gainesville, Fla., procedures for the impregnation of cotton and woolen uniforms with insect repellents by serial dipping were investigated. The best concentrations of deet, benzyl benzoate, and M-1960 and procedures for impregnation of wet and dry uniforms were established.

A gas chromatographic procedure was developed and used in an investigation of the amount of diethyl fumarate present in malathion concentrates or louse powders. The diethyl fumarate is a skin irritant.

4. Testing of Respiratory Protective Devices. An improved testing procedure was developed for assessing the safety of respirators for protection from toxic agricultural pesticides. Particles of pesticide in the size range encountered under practical conditions are injected into the air that enters the respirator. Gas chromatography is utilized to detect any pesticide that passes through the respirator cartridges or filters.

C. Methods of Analysis for Insecticide Residues.

A gas chromatographic method has been developed for the determination of residues of Union Carbide 20047A (3-chloro-5(or 6)-cyanobicyclo[2.2.1]-heptane-2-one N-methylcarbamoyloxime).

Two analytical methods have been developed for the determination of Imidan® (O,O-dimethyl S-phthalimidomethyl phosphorodithioate) residues in milk. One is a colorimetric method using chromatropic acid as a reagent and the other is a gas chromatographic method. The latter method has also been applied to analysis of corn plants.

A gas chromatographic method has been worked out for the determination of Zytron® (O-2,4-dichlorophenyl o-methyl isopropylphosphoramidothioate) residues in the tissues of chickens and in eggs.

A gas chromatographic method also has been perfected for determining residues of Shell SD-7438 (toluene alpha,alpha-dithiobis[O,O-dimethyl phosphorodithioate]) in sweet corn. The extraction, cleanup, and chromatographic behavior of the compound is similar to that of Imidan®.

An analytical procedure has been developed for residues of tetrachloro-, trichloro-, and methoxydichlorobenzoic acid in leaves, stems and roots of tomato plants. After conversion to the methyl esters the compounds are analyzed by electron capture gas chromatography. Response varies directly with the chlorine content of the molecules and 2-4 nanograms of the acids are required to produce a 50% response.

The extraction behavior of 133 different pesticides in 6 binary solvent systems has been studied and p-values determined for each. The p-value is the fraction of pesticide partitioning in the upper phase of the solvent system. It has been determined that the p-values of insecticides remain practically constant in the presence of food extractants and large amounts of other pesticides. This value is a useful guide in the extraction and cleanup of pesticides for residue analysis.

D. Aerosols for Insect Control.

At the request of General Services Administration changes were made in the specification for deet pressurized insect repellent spray. Recommendations included three aerosol valves without hermetical seals which could be used on this product. This should result in appreciable savings for the Armed Forces, which use large amounts of repellents.

Tests with commercially produced valves indicated that the specification for liquefied gas-propelled deet insect repellent called for too close a tolerance on the valve output rate. It has been proposed that a valve output of 0.75 gram minimum to 1.5 grams maximum per second at 80°F. be allowed instead of the present requirement of 1.0 $\pm$ 0.1 gram per second in the specification.

Water-based aerosols, now becoming commercially available, have two advantages over the regular oil-based type of liquefied-gas-propelled aerosols. The water-based aerosols are cheaper to formulate because of the low priced propellent used, and they contain a minimum amount of oil which results in reduction of the inhalation hazard. A disadvantage of water-based aerosols is that each time one is used it must be shaken to create a good emulsion. The propellent used is flammable. The shelf life has not been thoroughly established for most of the better water-based formulations and storage under freezing conditions has not been investigated. The particle size and biological efficiency are now about equal for the water- and oil-based types providing each is formulated properly and used with an appropriate valve. Tests carried out with several water-based aerosol formulations using a new commercially developed bleeder type valve showed that with this valve, particles in the aerosol range of 10 to 12 microns could be produced. With the valves previously available, the water-based formulations could not be atomized in the aerosol range. The new valve is constructed with a

bleeder hole which allows gas from the top of the can to mix with the liquid contents coming up through the dip tube. Water-based aerosols containing 3% of pyrethrum extract (20%), 1.4% piperonyl butoxide, 1.0% of emulsifier, 35.0% of an isobutane-propane propellent, 10 to 40% of distilled water, and methylchloroform to make 100% were tested against house flies in comparison with a oil-based formulation containing a comparable amount of active ingredients. The water-based formulations performed as well as the oil-based when the water content was 25% or less.

A new hermetically sealed aerosol valve with a low delivery rate has been tested and found satisfactory for application of the concentrated aerosol formulation that has been proposed for use by the military. This valve is produced by a new company which is not yet in regular production. The ability of the company to produce this valve on a large scale has not been determined.

A boom sprayer has been constructed to apply small volumes of malathion or other insecticide per acre. This machine utilizes a paint spray atomizer operating at 30 to 40 pounds per square inch. The nozzle is set to dispense 60 ml. per minute, and the malathion particles produced are in the aerosol size range. To prevent the particles from blowing away, the aerosol is released under a plastic cover that drags behind the tractor and forces the particles down beneath the foliage. A swath of 12 feet is covered and if the tractor is driven at 5 miles per hour about 1/2 pound of malathion is dispensed per acre.

Another new machine was constructed which holds two liquefied gas aerosol dispensers and a small weighted plastic canopy on a tubular frame. Each dispenser delivers 1 gram per second of formulation containing 3% malathion and 97% of propellent. This equipment has been used to apply malathion to bean plants at the rate of 1/2 lb. per acre or 1.6 grams per hundred foot row. The aerosols are directed at the underside of the bean foliage and they can be released mechanically by remote control. The apparatus is drawn by hand through the rows at speeds up to 3 m.p.h.

E. Biological Evaluation of Chemicals for Insect Control.

1. Insecticides. A major activity in this area is the laboratory testing of synthetic organic compounds and natural products against representative species of insects to determine whether the materials have insecticidal, synergistic, attractant, repellent, insect chemosterilant, growth controlling, or other effects that would be useful for insect control. Preliminary evaluation tests on these materials are carried out at Beltsville, Md., and Brownsville, Tex., by the Pesticide Chemicals Research Branch and at 21 other locations by other Branches of the Division, throughout the United States and in Mexico, on 65 insect and 8 mite species. Some of the materials

tested originate within the Pesticide Chemicals Research Branch and many others are supplied by other government or private research agencies and by industry. These materials are also submitted for evaluation to the Stored Products Insects Research Branch, Market Quality Research Division, laboratory at Savannah, Ga., in cooperative research with the Entomology Research Division.

A new reporting system on the results of these evaluations of candidate insecticides and acaricides was developed in 1964. Results received quarterly from each laboratory are compiled into a comprehensive report for internal use in the Department. In 1965 the report was broadened to include the results of laboratory evaluations on candidate attractants, repellents and chemosterilants. A total of 914 compounds were evaluated for insecticidal or acaricidal activity during the year. Most of these compounds were received from industry sources. Some were from the Pesticide Chemicals Research Branch or other government and university sources. A number of the compounds from industry showed considerable promise as broad spectrum insecticides in both laboratory and small plot field evaluations during the year. Preliminary data received indicate that some of these promising materials appear to have very favorable mammalian toxicities.

2. Materials That Control the Activities of Insects Through Effects Other Than Death. In the research on insect chemosterilants at Beltsville, Md., a study was made of the mating behavior of house flies and of the rapidity and duration of the sterilizing action of tepa on the male flies. One microgram of tepa injected into male flies reached 50% sterilizing effectiveness in 23 minutes and full effectiveness in about 3-1/2 hours. After one week partial restoration of fertility began to occur. In connection with this investigation, a bioassay method developed previously at Beltsville for evaluation of the potency of chemosterilants was greatly simplified and the labor involved was reduced by about one half without affecting the reliability of the assay. The metabolism of C^{14}-labeled tepa by male house flies was investigated.

Biological evaluations were made of two series of compounds related to tepa and hempa in a study of relationships between molecular structure and chemosterilant activity and acute toxicity toward male house flies (See B.1 for report of results). About 35 other compounds of different types were assayed for sterilizing potency at Beltsville. Many compounds also were screened at Brownsville, Texas, for chemosterilant activity.

In connection with investigations of insect sex attractants the study of the mating behavior of the American cockroach, Periplaneta americana, was continued. Information was obtained on the frequency of release of the sex attractant by females with respect to age, ovulation, fertility, temperature, and light-dark cycles and on the variability of the male display

before attractive females. Studies with cockroaches of the species P. americana and P. brunnea showed that there is a weak cross attractance between the sexes but no cross-copulatory behavior with the full display.

An electrophysiological study was made of the reception of odors by the antennae of male gypsy moths, utilizing electroantennograms. Stimulation response curves were determined for the synthetic gypsy moth sex attractants d-, l-, and dl-10-acetoxy-cis-7-hexadecen-1-ol, gyplure (12-acetoxy-cis-9-octadecen-1-ol), and a natural lure extract from this insect species. They were compared with each other and with the sexually neutral compounds n-amyl acetate, isoamyl acetate, hexane, and ammonia.

3. Aerosols and Space Sprays. Laboratory tests were carried out at Beltsville with a number of new insecticides and synergists in aerosols or space sprays to evaluate their effectiveness against resistant and nonresistant house flies or mosquitoes. New formulations of known insecticides also were tested.

In tests conducted at a practical dosage level in a 1000-cu. ft. test chamber, an aerosol containing 0.6% pyrethrins and 1.4% sulfoxide with xylene as solvent was as effective against resistant house flies and mosquitoes as the Federal Specification aerosol which contains 0.6% pyrethrins and 1.4% piperonyl butoxide. In other practical dosage tests an aerosol formulation containing 1% each of allethrin and dichlorvos was very effective against resistant strains of house flies and Aedes aegypti and Culex quinquefasciatus mosquitoes.

Tests were made with pyrethrin-piperonyl butoxide aerosols containing isopropyl myristate as an odor masking agent to determine whether the myristate adversely affected the insect kill. Concentrations of 1.5% and 3% did not reduce the kill, but 6% did.

In a study of the effectiveness of water-based aerosols tests were carried out with a water-based pyrethrum-piperonyl butoxide formulation against house flies in comparison with an oil-based formulation containing a comparable amount of the active ingredients. The water-based formulations were as effective as the oil-based one when the water content was 25% or less (See also section D).

In medical reports of experimental work on rheumatism it has been indicated that dimethyl sulfoxide in combination with certain pharmaceuticals greatly increases their penetration through the skin. Tests therefore were made with this compound in combinations with certain insecticides to determine whether it might increase their penetration through insect cuticle and thus result in greater effectiveness. In these laboratory tests dimethyl sulfoxide added to pyrethrins sprays caused no increase in kill of nonresistant house flies in direct spray tests. In tests on German cockroaches,

filter paper treated with chlordane plus dimethyl sulfoxide caused quicker knockdown of susceptible-strain roaches than chlordane alone. Neither was effective against resistant German cockroaches.

In spray tests against adult mosquitoes of different species Culex quinquefasciatus appeared to be more susceptible to pyrethrins than Culex pipiens and much more susceptible to allethrin. Aedes triserintus and Aedes albopictus were about as susceptible to pyrethrins as Aedes aegypti. Both species were more resistant to allethrin than Aedes aegypti.

Preliminary tests with the Official Test Aerosol against Periplaneta fuliginosa indicate that this cockroach is less resistant than other species of Periplaneta.

Tests were conducted with several sugar baits for control of face flies on herds of dairy and beef cattle in Maryland. One percent Ciodrin® (alpha-methylbenzyl 3-hydroxycrotonate dimethyl phosphate) alone or with 0.25% dichlorvos caused high reductions of face flies on dairy cattle treated daily for 14 days. Reduction generally remained high when treatments were made every second or third day. Dichlorvos 0.5% alone was not as effective as the Ciodrin sprays. Against beef cattle single treatments with 1% Ciodrin caused greater reduction of face flies than did 0.5% bromophos (O-(4-bromo-2,4-dichlorophenyl) O,O-dimethyl phosphorothioate).

F. Methods for Control of Insects in Aircraft.

1. Formulations. A series of comparative tests of aerosol formulations for aircraft disinsection were made at Beltsville in cooperation with the World Health Organization. In these tests a formula proposed by the Pyrethrum Board of Kenya, containing pyrethrum, sulfoxide, and isopropyl myristate, was compared with the WHO Standard Reference Aerosol and the G-1492 aerosol used for aircraft disinsection by the U. S. The Kenya formula was very effective but approval for aircraft use is not yet final.

Concentrated aerosol formulations containing (1) 10% allethrin or (2) 2.7% pyrethrins plus 6.3% piperonyl butoxide in 2-ounce containers were prepared for evaluation by the armed forces under combat conditions. These small containers of the concentrated formulations provide nearly as much insect killing power as the conventional 12-ounce containers of 0.6% pyrethrin aerosol now in use.

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AREA NO. 20. IDENTIFICATION OF INSECTS AND RELATED ARTHROPODS

Problem. Only about a third of the estimated two million or more kinds of insects in the world have been described and named. Many of these are of no known immediate concern to agriculture or mankind, but thousands of species are known to be or are potentially destructive or useful. Minute morphological differences are very important in recognizing many species, and only highly trained specialists are able to positively identify known species and describe new ones. Precise information on the identity and distribution of insects is essential to the efficient conduct of programs concerned with research on harmful insects and the development of methods for their control, and in the management of regulatory activities intended to exclude, control, or eradicate insect pests.

Knowledge of the classification and identification of insects at present is far from adequate. Knowledge of the insect fauna of the world provides the best assurance that any potential pests will be immediately recognized, so that appropriate safeguards can be set up to exclude them or prompt action taken to control or eradicate them if accidentally introduced. Moreover, with increasing emphasis on the utilization of beneficial insect parasites and predators to help control destructive insects, it is necessary that we know which insects to search for, where they might be found, and how to recognize those that may be useful.

USDA AND COOPERATIVE PROGRAM

The program of the Department is a long-continuing one involving insect taxonomists. It includes basic research to make known to science previously unrecognized and undescribed species of insects, ticks, and mites, and the application of the results of this research to the problems of insect identification. The work is carried on to a limited extent at Beltsville, Md., but mostly at two locations in Washington, D. C., in close cooperation with the U. S. National Museum of the Smithsonian Institution. Cooperation, close but somewhat less active, is maintained with various centers of taxonomic research in the United States and in foreign countries, and with numerous individuals in many parts of the world.

The Federal scientific effort devoted to research in this area totals 31.0 professional man-years. Of this number, 8.9 are devoted to basic studies to name and describe beneficial and injurious insects, mites, and ticks; 12.2 to the identification of insects, mites, and ticks; 8.4 to the preparation of keys and monographs on the classification, distribution, morphology, and biology of insects and related arthropods; and 1.5 to program leadership.

In addition Federal support of research in this area by means of 2 grants and 1 contract provides 0.8 man-years devoted to basic studies to name and describe beneficial and injurious insects. One of the grants is to Cornell

University for basic studies on the taxonomy, morphology, and ecology of cutworm larvae and the other is to North Carolina State of the University of North Carolina for basic studies on the nature and taxonomic significance of morphological characters of female leafhoppers. Research on the contract comprises a taxonomic study of the North American weevils related to the boll weevil, and of various populations of the boll weevil itself, by the Agricultural Experiment Station of Texas A&M University.

Research in this area is also conducted under eight P. L. 480 projects. In Uruguay S9-ENT-6 provides for 1.5 professional man-years devoted to the classification of grasshoppers, and in Colombia S5-ENT-2 has 2 man-years devoted to a biochemical study of Drosophila classification. Four projects are operating in India as follows: A7-ENT-24 provides 3 professional man-years for a systematic study of thrips; A7-ENT-28 provides 2.25 man-years for taxonomic studies of Mallophaga (biting lice); A7-ENT-29 provides 2 man-years for a taxonomic study of Bruchidae (seed beetles); and A7-ENT-37 approximately 2.5 man-years for a taxonomic survey of parasitic Ichneumonidae in India. A project in Egypt (F4-ENT-2) provides 4 professional man-years for a study of the insect fauna of Egypt. In Pakistan A17-ENT-10 provides 1.5 man-years on leafhopper taxonomy.

PROGRAM OF THE STATE EXPERIMENT STATIONS

The State stations in general each maintain a collection of preserved specimens of insect pests and beneficial insects that are of economic importance in the State. They also maintain a general collection of insects which may contribute to taxonomic research. Both of these collections are used in the identification of insects that are collected in the State. At a number of State stations the work in insect taxonomy is combined with ecological and biological studies. This combines the information on the physical characteristics of the insect by which it is identified with information on behavior including emergence, mating, flight, host selection, oviposition, feeding, activity patterns and other information and often includes data on various stages of the insect.

The number of insect species is so large that no one individual can be familiar with all of them. In most instances, an individual is familiar with only a limited group. At each of the state stations the work on taxonomy is usually limited to one or two groups which are of special interest to members of the staff. In one instance, taxonomic and biological investigations are conducted on small parasitic wasps--Chalcidoidea. Taxonomic analysis and revision of this group are being based on morphology, host relationships and behavior. Microlepidoptera are being studied in a similar manner. In Hawaii, taxonomic research emphasizes the fruit flies of the Pacific and Oriental regions. In other states, neotropical mosquitoes, aphids, scale insects and leafhoppers are being studied. In addition to the taxonomy of insects, some state stations are doing taxonomic work on mites with the work mainly on mites of economic importance in the state. Studies are being made of the chromosomal evolution in insects. The identification

of insect pests is an important aspect of the survey of economic insects which is carried on annually by a number of states, usually in cooperation with U.S.D.A.

A total of 25.8 professional man-years is devoted to this area by the state stations.

PROGRESS - USDA AND COOPERATIVE PROGRAM

A. Basic Studies to Name and Describe Beneficial and Injurious Insects, Mites, and Ticks.

1. Mites. Certain mites are beneficial in being predators on injurious insects and mites. Two new species of such mites, belonging to the family Erythraeidae and preying upon eggs of the cotton bollworm, have been described.

As contributions to our knowledge of African mites, some species of Tarsonemidae, and a new species of Fusoherecia, have been described and named.

2. Two-winged Flies. Several genera in various Diptera families have been under revision for a number of years. New species in these genera have been described and additional observations on taxonomic characters and the nomenclature of several species already known to science have been published.

Studies on the mating behavior of certain species of vinegar flies which are closely related have been conducted in Colombia under the P. L. 480 program. They show how sexual selection by mating males and females affect the evolutionary patterns of the species. These findings will be used later to help trace the evolution of taxonomic characters.

Studies of certain type specimens in the fly families Chloropidae and Tephritidae were undertaken to resolve problems of species identification that have existed for years.

3. Grasshoppers. In cooperation with a California entomologist, several new species of Melanoplus, a genus that contains grasshoppers of considerable economic significance, were discovered and named.

Well over 7,500 grasshoppers and their close relatives (family Acrididae) have been collected in Uruguay and portions of neighboring countries to form material for a general taxonomic study of that group being undertaken under P. L. 480. The principal value of these collections lies in the fact that many of these specimens were found in the same localities as some which have been previously, but poorly, described by previous taxonomists.

4. Moths. A new genus and species of Oecophoridae, a family of small moths at lease one species of which attacks stored products, were found in Florida

and described as new to science. A new Cuban species of Iodopepla, a cutworm genus, was also described. A change in the generic name of the fall armyworm was announced as a result of recent taxonomic studies on the genus.

5. Hemipterous Insects. Some new species of leafhoppers from North, Central and South America, some related to those that inflict serious crop damage, have been brought to light; taxonomic notes on two species of toad bugs were published; and a new Florida species of white fly and observations on two others were discussed in print. The marine water strider genus Hermatobates, was discovered for the first time in the Atlantic Ocean, and a new species was recorded.

6. Parasitic Wasps. A new species of the genus Dendrosoter from the Philippine Islands has been named.

7. Thrips. A species of very small desert thrips was discovered to deserve generic status of its own, thus further straightening out the taxonomy of the genus Anaphothrips. The previously undescribed male was also brought to light and its characters recorded.

As a result of work done under the P. L. 480 program, a significant amount of study material has been accumulated which will facilitate future taxonomic studies on Indian thrips. New genera and species of Indian thrips have been described, and taxonomic notes on others have been published.

8. Book-lice. A new genus of Neotropical psocids, with unusual horn-like structures on the head, was discovered and named. This species, like most others in the order Psocoptera, occur in nature on the bark of trees and do not infest households at all.

9. Beetles. As a result of a P. L. 480 grant, 20 species of seed beetles (Bruchidae), 9 of which are recognized as economically important, have been collected in the Punjab and neighboring states in India. The immature stages of 14 of these species have so far been found. Surveys for these beetles are continuing to the end that the various species occurring in the Punjab may be easily identified in both the adult and immature stages. Important host records for all the species so far seen in the study have also been obtained.

10. General. The insects of Egypt are being surveyed in a cooperative P. L. 480 project in which USDA scientists are participating. Since the inception of the project, about 15,000 specimens representing all the insect orders have been collected and are being mounted, labeled, and studied. A number of specimens have been sent to participating specialists for identifications, and a list of the Diptera now known to occur in Egypt has been prepared.

B. Identification of Insects, Mites, and Ticks.

Authoritative identifications and references to pertinent taxonomic and biological literature are supplied to support Federal and State research,

control, and regulatory activities pertaining to entomological problems. These services are also performed for industry, pest control operators, and private individuals in the United States, and for foreign agencies and institutions concerned with entomology.

During the year, a total of 31,477 lots of insect material was received for identification. Well over 251,000 specimens were examined. A total of 68,860 identifications was made and reported. Specimens were accepted for identification only when rendering the service could be justified, since there is a backlog of material awaiting study.

The sources of material and the numbers of identifications made of the specimens received from each are shown in the following table:

Source	Number of Identifications	Percent of Total
Agricultural Research Service		
Plant Quarantine Division	21,199	30.76
Plant Pest Control Division	3,053	4.44
Entomology Research Division	4,173	6.06
Forest Service	1,638	2.38
Agricultural Marketing Service	195	0.28
Other Federal Agencies	2,365	3.44
States and Insular Possessions	20,417	29.66
U. S. individuals	8,264	12.01
Foreign agencies and individuals	7,556	10.97
Total determinations	68,860	100.00

Many of the specimens received for identification are of much interest, either representing new species not previously in the National Collection, or documenting new distributional data. For these reasons, 35,455 specimens of especial value were added to the National Collection during the year.

The systematic review of technical literature essential to the programs in this area included the examination of 2,182 publications which contained 5,867 articles of interest to insect taxonomists. Reference (by author) cards to these articles totaled 8,355. A total of 2,377 articles was cataloged in depth, and from this effort 34,852 file cards were made up on which data of significance to taxonomists were recorded. The cards are in continual use in research and service activities, and the file for each specialist is kept up to date and immediately available to him.

During the year 98 visitors obtained aid on taxonomic, nomenclatural, and other problems. The visitors remained for varying periods of time, from an hour or so to several weeks, and came from all parts of the world.

C. Preparation of Keys and Monographs on the Classification, Distribution, Morphology, and Biology of Insects and Related Arthropods.

1. Mites. Two major contributions to knowledge of the classification of mites treat the Spinturnicidae of Southeast Asia and the Pacific Region and the Phytoseiidae of Central America. Each treatment contains full illustrations of the anatomical characteristics by which the known species may be recognized.

2. Wasps. The pteromalid wasp genus Metacolus, some species of which parasitize scolytid beetles in forest trees, was reviewed. This work contains keys for the identification of the various species, and notes on their description and known biologies.

Studies on the biology of various North American solitary wasps were continued from previous years, yielding a great deal of additional information about the bionomics and prey of many species, principally in North Carolina and Florida.

3. Ants. Species of ants are widely known to infest households in the United States, many of them causing much annoyance by hunting out food-stuffs in kitchens and by causing painful itchings and swellings by their bites. The house-infesting ants of the Eastern United States have been reviewed in a Technical Bulletin. Each of the species discussions includes a brief description and illustration, a statement about the biology and economic importance of the species, and references to other publications where further information can be found.

4. Two-winged Flies. Keys, distributional notes, and taxonomic and nomenclatural observations have been made on a number of fly families which at present are the objects of rather intensive systematic study. The genera so reviewed are Monohelea in the Neotropical Region and Alluaudomyia of the Oriental Region (biting midge family Ceratopogonidae), the genera of the shore fly subfamily Parydrinae, the platystomatid genus Poicilotraphera, the tephritid genus Xanthomyia, and the genera of black flies occurring in Micronesia. In addition, the hosts of the parasitic tachinid tribe Eutherini have been reviewed.

Systematic studies of two families, the black flies (Simuliidae) and the snail flies (Sciomyzidae), have been conducted to gain a more thorough understanding of the relationships of their respective genera.

A three-month survey of flies on the island of Dominica, B.W.I., was undertaken in cooperation with the National Museum of the Smithsonian Institution. Over 19,000 specimens of Diptera were collected, pinned, and labeled for further study. The survey revealed that (a) there are probably more species of Diptera, and more individuals, in Dominica than all other insects combined; (b) lake and stream habitats are poorly utilized by flies, but wet leaf-litter, moss, bark, and debris in leaf axils and rot holes yield large

numbers of flies of many kinds during the dry season; and (c) the large, conspicuous flies are absent from the island fauna.

5. Beetles. The Bruchidae are beetles that develop in the seeds of plants. Species of the bruchid genus Neltumius have been keyed and discussed in order to facilitate their identification. A study of a fossil bruchid compares its relationship to modern genera in a systematic study.

A three-month survey of certain beetles on the island of Dominica was undertaken, also in cooperation with the National Museum (see C4 above). The beetles sought were those living in the wood of felled trees, especially those of the rain forests. The dominant trees, Sloanea spp. and Dacryodes spp., yielded many interesting scolytids, cerambycids, and weevils, and small beetles were especially abundant in bromeliads and fungi associated with these trees.

6. Leafhoppers. A review, containing illustrations, keys, and descriptions, of the species of the African leafhopper genus Wolfella was completed, and several genera and species were reviewed to supplement and update some of the published works on leafhoppers of the American tropics.

Under P. L. 480, 20 genera and 54 species of leafhoppers have been recorded for the first time from Pakistan; most of the species are previously undescribed. The survey is the first one ever made of leafhoppers in the particular area, which lies on the western border of the Oriental Region in the Indian subregion.

7. Thrips. In cooperation with a Canadian entomologist, the genus Taeniothrips in Canada was revised. This work brings together all of the currently available information on this flower-infesting genus as it occurs in Canada.

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AREA NO. 21. FOREIGN EXPLORATION, INTRODUCTION AND EVALUATION OF BIOLOGICAL CONTROL AGENTS

Problem. Many of the most serious insect and weed pests in the United States have been accidentally introduced from foreign countries without the insect enemies that kept them under control in their native homes. Some of the harmful insects so introduced have been effectively controlled by later introduction of their parasites and predators. Foreign exploration for beneficial biological control agents of insects and their subsequent introduction, colonization, and evaluation in this country is now a well established practice in the control of introduced insect pests. The use of imported insects to control introduced noxious weeds, although a more recent practice, has shown much promise. The biological approach to the control of insect and weed pests has great potential. Therefore, further foreign exploration is needed and additional research is necessary on the biology, ecology, nutritional requirements and the most effective manner of utilizing natural control agents, if they are to be used to maximum advantage. There is growing concern by the public over the insecticide and other residue problems in foods and by conservationists over the potential hazards of insect control chemicals to fish and wildlife. More effective use of natural control agents in meeting destructive insect and noxious weed problems could materially contribute to the ultimate objective of overcoming the pesticide residue and other hazard problems associated with the use of chemicals for the control of insects and weeds.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing program on the use of beneficial insects. Basic and applied research is conducted on insect parasites and predators of insect pests and on insects that attack weeds, including foreign explorations for beneficial species and their introduction, liberation and evaluation in this country. A laboratory is maintained at Nanterre (near Paris), France, for studies on the parasites and predators of agricultural pests that have accidentally been introduced from Europe into the United States. At a station in Rome, Italy, studies are in progress on insects attacking a number of weeds, including Scotch broom, tansy ragwort, Dalmatian toadflax, Mediterranean sage, Russian knapweed, and halogeton. Seasonal studies were carried out during the year at appropriate localities in the Soviet Union where surveys were conducted on insects attacking halogeton and puncturevine and on parasites and predators of the alfalfa weevil and the cereal leaf beetle. Research on insects affecting aquatic weeds, especially alligatorweed and water hyacinth, is being conducted at the National Agricultural Research Center in Castelar (near Buenos Aires), Argentina. In the United States a receiving station and laboratory is maintained at Moorestown, N. J., where major emphasis is given to receiving, propagating, and transshipping insect parasites to proper liberation points. A laboratory for receiving, studying, and liberating insects affecting range weeds is located at Albany.

Calif. A long-range program to include numerous aspects of biological control of insect pests has been initiated at Columbia, Mo. Studies regarding entomophagous insects are also conducted at Riverside, Calif. The work at Albany and Riverside is conducted in cooperation with the University of California and the California Experiment Stations, and the work at Columbia is conducted in cooperation with the University of Missouri and the Missouri Agricultural Experiment Station. Four grants and 2 contracts for domestic research have been executed that are concerned with study of insect parasites and predators. The grants are to Washington State University, the University of Missouri, the University of Arkansas, and the University of Minnesota. The contracts are with Purdue University and the University of California at Riverside. One grant and one contract are with Louisiana State University and the University of Idaho, respectively.

The Federal scientific effort devoted to research in this area totals 23.0 man-years. Of this total, 2.9 is devoted to search for and importation of foreign parasites and predators of insect pests; 2.3 to search for and importation of foreign insect enemies of weeds; 12.9 to basic biology, physiology, nutrition and evaluation; 3.9 to receipt, liberation and establishment of foreign insect enemies of insect pests and weeds; and 1.0 to program leadership.

In addition Federal support of research in this area conducted under grants and contracts provides for a total of 2.9 professional man-years. Of this total 0.9 is devoted to search for and importation of foreign parasites and predators of insect pests; 1.4 to basic biology, physiology, nutrition and evaluation; and 0.6 to studies of native insects that attack weeds of foreign origin.

Thirteen grants from P. L. 480 funds providing for 47.5 professional man-years have been executed for projects directly concerned with the study of insect parasites and predators. Nine of these projects involve exploration for beneficial species that might be shipped to the United States for trial and release against agricultural pests here.

Grants for three P. L. 480 projects providing for 8 professional man-years have been executed for studies on the biological control of weeds.

PROGRAM OF STATE EXPERIMENT STATIONS

A good research program is in progress at the State stations to identify and determine the distribution and abundance of parasitic and predatory insects that feed on agricultural insect pests and mites. In these studies, biological and ecological data are gathered on these beneficial insects and their usefulness as a means of control is evaluated. Studies are made of entomophagous insects that feed on the insect pests attacking alfalfa, olive, cotton, tobacco, sweet corn, vegetable, greenhouse and other crops. A number of aphid pests have been imported without bringing their natural enemies with them. Parasite and predator introductions may establish

biological control. Basic information on parasites and predators of forest insects is being obtained so that methods and techniques for biological control can be developed. The parasites of the green stink bug, which was recently found in Hawaii, are being studied.

Research is in progress on the influence of climatic factors on growth reproduction, survival and behavior of parasites and predators on selected insect pests. In general, this work has been discussed in the report of research on the particular commodity involved. Climate often influences or modifies the efficacy with which natural enemies control the abundance of pests. Nutritional studies are made on parasites and predators and their host to promote more efficient mass production and utilization. Strip harvesting and other means of maintaining parasite and predator populations in nature are under investigation. Identification and classification of insect parasites and predators are being studied. By determining which insecticides may be harmless to or have the least detrimental effect on parasites and predators, an integrated control program may be developed whereby both chemical and biological control are utilized to supplement each other.

A total of 26.3 professional man-years is devoted to this area of research by the State stations.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Search for and Importation of Foreign Parasites and Predators of Insect Pests.

1. Parasites and Predators. Eighteen species of parasites or predators of 7 kinds of insect pests were collected in Europe for introduction into the United States, and 26 species of parasites or predators of 6 kinds of insect pests were sent to the United States from P. L. 480 projects in India and Spain. All of these beneficial insects, approximately 49,000 specimens in about 300 shipments, were received by the Moorestown, N. J., laboratory for screening, testing and transshipment of living material to liberation points throughout the United States or to U. S. Department of Agriculture Field Stations or State Experiment Stations for further testing and propagation before liberation. Seven beneficial species were collected or reared from laboratory stocks at Moorestown and 71 shipments (nearly 64,000 specimens) made available to State or Federal Laboratories for release and future evaluation. Some 48,500 of these specimens shipped belonged to a single species, Tetrastichus incertus Ratz., a promising parasite of the alfalfa weevil. Shipments of this species were made to 13 States.

2. Uruguayan Parasites of Insect Pests. Surveys for and biological studies of parasites that attack insects of importance to United States agriculture are being conducted in Uruguay under a P. L. 480 Grant. Parasites, predators or pathogens of such insects as the fall armyworm, corn earworm, sugarcane borer and potato leafhopper have been discovered. Information from this

research is of potential value in programs aimed at introductions of beneficial insects into the United States.

3. Parasites of Brazilian Plant-feeding Insects. A comprehensive catalogue of the plant-feeding insects of Brazil with data on their biologies and records of parasites and predators that attack them, is nearly complete. In connection with preparation of the catalogue under P. L. 480, a bibliography of Brazilian entomology through 1962 has been compiled. The bibliography is being prepared for publication and will be issued before the catalogue is printed.

B. Search for and Importation of Foreign Insect Enemies of Weeds.

1. Enemies of Weeds. No new species of insects that attack weeds were released by Entomology Research Division personnel in the United States during the year. Living material of 2 kinds was received at Albany, California, from Europe and these species are being given final host specificity tests before recommending liberation. One of these is a moth, that attacks halogeton, sent from Spain in July 1964, the other a seed destroying fly that attacks tansy ragwort, specimens being sent in January 1965 from material collected in France. Host plant specificity tests are being conducted with a number of promising insects that feed in Pakistan on weeds that also occur in the United States. Collections were made of 3 weed-feeding insects originally from Europe and established in California. One of these species was sent to additional localities in California, one to Washington, the third to Hawaii.

C. Basic Biology, Physiology, Nutrition, and Evaluation.

1. Cereal Leaf Beetle. Studies of biology of the several species of parasites of the cereal leaf beetle were continued in Southern France and in Italy. Particular emphasis was placed on an egg parasite, Anaphes flavipes (Foerster). The parasite was collected in West Germany, France, and Italy, and a wide geographical distribution of the species is apparent. Adults reared from leaf beetle eggs collected during May in the field at Rome, Italy, were confined with additional eggs and oviposition obtained. The parasite is attracted to eggs from a distance of 2 or 3 millimeters. Beetle eggs that are less than one day old or in high humidity are sticky and female parasites become entangled in this adhesive covering. At temperatures ranging from 65° to 75° F development of Anaphes from oviposition to emergence required 10 to 12 days. Mating by the parasite occurred immediately after emergence of the adults and adults live about 4 days. The number of adults issuing from a single host egg varied from 1 to 7, the typical number being 3 of which 1 is regularly a male and 2 are females. Attempts to find a late season host for Anaphes were not successful although females readily oviposited in unidentified chrysomelid eggs on the plant Polygonum aviculare.

2. Alfalfa Weevil. Research on factors that control diapause of this insect was continued in an effort to discover means of breaking diapause of Microctonus aethiops, a parasite of adult weevils. None of the regimens to which diapausing weevils were subjected was notably effective in producing adult parasites from the first stage larvae in the weevils. However, the greatest number of parasites emerged from weevils stored at 35° F and 10-hour photoperiod over a period of 16 weeks.

3. Bathyplectes anurus. This parasite of larvae of alfalfa weevils was subjected to initial tests against the Egyptian alfalfa weevil, Hypera brunneipennis, in California. Twenty-five adults of the parasite, received from Moorestown, New Jersey, were used in successive tests with about 1800 Hypera larvae, and 731 cocoons of anurus resulted. This represents a yield of approximately 43 cocoons per female parasite and shows that anurus can develop readily in this new host.

4. Natural Enemies of Lygus Bugs. Predacious bugs of the genus Geocoris are among natural enemies of Lygus bugs in the Southwest. Studies reveal that nymphs of species of Geocoris are subject to attack by a parasitic fly, and eggs of Geocoris are hosts for a parasitic wasp. These parasites, and probably other undiscovered ones, are responsible for the unsatisfactory rate of predation by Geocoris.

5. Natural Enemies of Rice Pests. A comprehensive, final report on the parasites or predators that attack rice pests in India includes information on biology and distribution of 57 rice insects. Most of these insects are moths the larvae of which bore in rice stems or feed on rice foliage. Large numbers of parasites were found, many of the records being of species not previously known from India or not known from the particular rice pests. Important biological data on some of the parasites have great potential value to entomologists working on them in the United States.

More than 120 species of insects have been found associated with rice in Pakistan. At least 20 of these species are injurious to rice. Fifteen kinds of parasites of rice insects have been found in Pakistan and rather low rates of parasitism recorded. Studies were made on susceptibility of rice varieties to attack by stem borers. The variety Sonhari Kangni was free of borer attack whereas other varieties suffered as much as 45% damage.

6. Natural Enemies of the European Corn Borer and Heliothis. Accounts of biology and techniques for rearing numerous Indian parasites of the indicated pests are included in an interim report of research being conducted in India under P. L. 480.

Research on natural enemies of corn borers is also being conducted in Pakistan where parasitism of the principal borers is found to be low. The so-called European corn-borer has not been found attacking corn in Pakistan but confines its feeding to a thistle, Cnicus wallichii. Two kinds of parasites were reared from the borer.

7. Tansy Ragwort. Tests are being conducted in California under quarantine conditions to determine the specificity of a European species of fly that destroys ragwort seeds, and, if released in the United States, would reduce production of seed by this plant. Tests on 3 species of Senecio native to North America and belonging to the plant genus to which ragwort belongs, are apparently conclusive enough to warrent a pending recommendation that the fly be liberated in the Western States.

8. Alligatorweed. Additional releases of a South American flea beetle that confines its attack to alligatorweed were made in the vicinity of Jacksonville, Florida, and Gulfport, Mississippi. The colony released in March 1964 on the Savannah National Wildlife Refuge, South Carolina, has increased in numbers and appreciable damage to alligatorweed has been observed. A second South American insect that feeds on alligatorweed, a thrips, has been tested and found to be unable to develop except on the weed.

9. Other Weeds. Considerable progress has been made on a study, in Pakistan, designed to discover insects that may be imported into the United States as biological agents to suppress noxious weeds, e.g. halogeton, dodder, cocklebur, and nutgrass. A moth whose larvae attack halogeton in Baltistan is being tested to determine if it feeds on useful plants. Studies are being conducted to determine host-specificity of a two-winged fly that lives on dodder and moths that attack cocklebur and nutgrass. Insects that feed on witchweed, and on aquatic weeds such as water hyacinth, are being studied in India. A stem-feeding weevil and a seed-feeding moth have been discovered associated with witchweed and these insects are being studied further in order to assess their potential for suppressing witchweed.

10. P. L. 480 Projects. A survey throughout India for parasites and predators of insect pests that attack grains, forage, oil-seed plants, vegetables, deciduous fruits and citrus is being conducted under a P. L. 480 Grant. Lists of parasites and predators have been developed from data gathered in field studies. Additionally, specimens of some Indian species of insects have been submitted for inclusion in the National Collections of Insects.

Surveys for natural enemies of 5 important species of aphids are being conducted in India and Taiwan, with support from P. L. 480 Grants. Neither project has been in force long enough to gather data useful to a program of introductions and releases of biological agents in the United States. Promising parasites and predators have been discovered in India, however, and releases of them in the United States will be given consideration soon.

Methods for large-scale rearing of parasites that attack such pests as Heliothis spp., Prodenia, and Spodoptera are being investigated under a P. L. 480 Grant to the Institute of Agriculture, Anand, India. Techniques on culturing host insects, using natural foods and artificial media, are being worked out before an attempt can be made to produce large numbers of parasites.

Methods for controlling the coconut rhinoceros beetle, Oryctes rhinoceros L., are being investigated at the Central Coconut Research Station, Kayangulum, Kerala, India, under a P. L. 480 Grant. Insecticidal treatment of manure piles gave controls of larvae from 80% to 100%. Data on the predators and microorganisms associated with the beetle are being gathered.

D. Receipt, Liberation, and Establishment of Foreign Insect Enemies of Insect Pests and Weeds.

1. Cereal Leaf Beetles. Three parasites of the cereal leaf beetle were collected in Europe, special attention being given to a promising species that destroys eggs of the beetle. Nearly 200 specimens of this species of minute parasite were supplied to cooperators in Michigan and Indiana for propagation and release when sufficient numbers are obtained to make this practicable. About 300 specimens of the leaf beetle larval parasite Tetrastichus julis and smaller numbers of 2 species identified as Tersilochus sp. and Hyposoter sp. were imported and shipped to Indiana for propagation and liberation. About 175 specimens of Tetrastichus julis, 175 of Tersilochus and a few Hyposoter were released in Indiana in May and June.

2. Alfalfa Weevil. Sixteen shipments of 4 species of alfalfa weevil parasites were received from France and shipped to cooperators for release in Missouri, Massachusetts, Pennsylvania, New Jersey, Delaware, Maine, Ohio, Indiana, and California. Among these parasites were over 600 specimens of Necremnus leucarthros, a wasp reared from pupae cells of Oulema gallaeciana, that may effectively attack alfalfa weevil larvae in their cocoons.

3. Face Fly. Two shipments of a staphylinid beetle that parasitizes puparia of the face fly were sent to the Division's laboratory at Lincoln, Nebraska, for study and propagation before release in face fly infested areas.

4. Grasshoppers. A modest number of 2 kinds of parasitic flies, reared from grasshoppers in France, were sent to the Division's laboratory at Bozeman, Montana. There were 16 adults of Blaesoxipha lineata and 18 of Acridomyia sacharovi in the shipments. Laboratory propagation of these species is to be attempted in order to determine their ability to breed in North American grasshoppers.

5. Natural Enemies of Lygus bugs. Nearly 700 living specimens of a parasitic two-winged fly were collected in the Eastern States, where it is known to parasitize Lygus, and shipped to Southern California as a possibly effective agent to reduce damaging populations of Lygus bugs.

6. Miscellaneous Insects. Several thousand eggs of the European predator Coccinella septempunctata were obtained from a living colony in the laboratory at Moorestown, N. J., and the eggs as well as fertile adults were distributed to cooperators, both State and Federal, in Maine, Washington, Ohio, Pennsylvania, and Delaware. Five shipments of Collyria

calcitrator, a parasite of the wheat stem sawfly, were sent from New Jersey to the Division's laboratory at Bozeman, Mont., in an attempt to establish the parasite there. In continuing cooperation with entomologists in Europe, 1257 specimens of North American predator Elatophilus inimica were collected in Connecticut by Forest Service personnel and sent to France through the Moorestown laboratory for release against a pine-destroying Matsococcus scale insect.

7. Puncturevine. The two stem-boring and seed pod-infesting weevils, previously established and multiplying in the Western States, have been released in Hawaii and are responsible for destruction of 2 species of the weed-genus Tribulus over large areas.

8. Tansy Ragwort. The cinnabar moth, Tyria jacobaeae, first imported from France and released in California in June 1959, has built up very high populations in the Fort Bragg, California, area. Adequate material is now available to make large releases in Washington and Oregon.

9. Scotch Broom. Approximately 3,000 cocoons of the Scotch broom twig miner, Leucoptera spartifoliella, were collected at Mill Valley, California, on April 27, 1965, and shipped to Humboldt County, California, for release. In May, a crew of men cut a pick-up truck load of broom twigs infested with the miner, for release in Placer County, California.

10. P. L. 480 Projects. Five shipments of parasites of the European corn borer, reared in India under P. L. 480 Project A7-ENT-9, were received at Moorestown, New Jersey, and transshipped to the Division's laboratory at Ankeny, Iowa. The shipments totaled 140 specimens of Scambus sp., and 38 specimens of Bracon sp. Receipts of parasites of Heliothis spp., from India under the same P. L. 480 Project were extensive and specimens of about 10 species were sent to the Division's laboratory at Tifton, Georgia, for propagation and possible release. Parasites of the sugarcane borer, collected in conduct of research under P. L. 480 Project A7-ENT-1 (India) and not reported previously, were sent to the Division's laboratory at Canal Point, Florida. Included were Iphiaulax sp., Stenobracon dessae, S. nicevillei, Apanteles sp., and Campyloneurus mutator.

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AREA NO. 22. INSECT PATHOLOGY

Problem. Basic investigations on viruses, fungi, bacteria, nematodes and protozoa are needed to fully exploit the use of such microorganisms as an approach to insect control. There is much interest in the use of these natural insect-control agents to overcome the growing concern over chemical residues following the application of insecticides to agricultural crops and livestock, and the increasing resistance of some insects to certain insecticides. The utilization of pathogens to produce diseases in insect populations, and so reduce them and the damage they cause, is an approach that has already shown great promise. Microorganisms that are pathogenic for insects are generally very efficient when used properly. They are specific for their insect hosts and harmless to men and other vertebrates. Basic research is needed for a thorough understanding of insect pathogens, including their growth and nutritional requirements, their resistance to environmental factors, and their mutability and mode of action, both in the laboratory and the field. Such knowledge must be obtained before these organisms can be used effectively in the control of insect pests.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing basic research program on the growth, nutritional requirements and mode of action of viruses, bacteria, and nematodes affecting insects. At the Pioneering Research Laboratory on Insect Pathology at Beltsville, Md., studies are in progress on mutability-induced changes in virulence of insect diseases, and the resistance of insects to diseases, including studies of the effect of the environment on the pathogens. A comprehensive reprint library on insect pathology is being assembled. Collections of all spore formers and viruses known to cause disease in insects are being obtained from world-wide contributors. A service involving the diagnosis of unhealthy insects is now available to Division, State, and University laboratories.

The program includes collaborative studies with the Pesticide Chemicals Research Branch on instrumentation for monitoring insect activity, internal temperatures of insects, and effect of gaseous atmosphere on metabolism and development of insects. Collaborative studies are also under way with the Pioneering Research Laboratory on Insect Physiology on the effect of microorganisms on insect sterol requirements. A cooperative project to study the progress of the non-inclusion virus disease of the citrus red mite, through electron microscopy, has been set up with the Fruit and Vegetable Insects Research Branch. A second cooperative study on the serology of this virus is under way in cooperation with the Insect Pathology Research Institute, Sault Ste. Marie, Ontario, Canada A contract was negotiated with the Rosner-Hixson Laboratories for studies on mammalian

toxicity and pathogenicity of insect viruses. Contracts were also negotiated with Ohio State University, Rutgers University and University of Maryland for research on mass production of virus diseases of four insects of major economic importance.

A cooperative project has been conducted with the Fruit and Vegetable Insects Research Branch to study the serology of the non-inclusion virus of citrus red mite, in order to develop a tool for diagnosis of the disease. Cooperative studies on the identification of B. thuringiensis varieties are being carried out with the Institute of Pasteur, Paris, France.

Federal scientific effort devoted to research in this area totals 9.0 professional man years. Of this number 3.5 is devoted to virus diseases of insects; 3.6 to bacterial protozoan, and fungus diseases of insects; 1.0 to nematodes and their associated bacteria pathogenic to insects and 0.9 to discovery and study of new pathogens.

In addition Federal support for 2.2 professional man-years devoted to research on virus diseases of insects is provided under contracts.

Additional research is in progress under P.L. 480 funds at The Institute of Plant Protection, Poznan, Poland, 1.5 man-years) and at the Annamalai University, Madras, India (3.0 man-years).

PROGRAM OF THE STATE EXPERIMENT STATIONS

The research on the diseases of insects and methods of using these to control pests is active at the state stations. The diagnosis of insect diseases including the development and improvement of methods, techniques and processes, the systematizing, cataloguing and analyzing of symptoms, pathological changes and diagnostic procedures are being studied. The possibilities of in vitro cultivation of insect viruses by means of tissue and cell culture, the phenomena of virus infection, and the chemical and physical properties of insect viruses are being studied. Basic knowledge is being gained relating to the cause, pathogenesis, and pathology of infectious diseases of insects. The properties and characteristics of the pathogens -- their virulence, infectivity, survival and dispersal capacity; the properties and characteristics of the host populations -- their susceptibility, resistance and methods of transmission; the effect of environmental factors on the pathogen and host; and the factors which govern the form and shape of the epizootic curve and the initiation, development and cessation of an outbreak of disease among insects are being studied. Means of mass production and application of naturally occurring pathogens are being developed. Native and introduced pathogens including commercially produced materials are being disseminated and evaluated for their effectiveness in the field. In general, these specific studies are included in the report of research on the particular commodity involved.

Optimum temperature requirements for pathogens of insects are being determined. Milky disease is being studied to determine its effectiveness in the control of Japanese and oriental beetles and the physiology of its sporulation and germination.

A total of 22.5 professional man-years is being devoted to this area by the state stations.

PROGRESS -- USDA AND COOPERATIVE PROGRAM

A. Virus Diseases of Insects

1. Virus Production and Infectivity Tests. As host insects have become available, viruses have been propagated in them, purified, and stored. The following infectivity tests were conducted. Against Prodenia eridania (Cramer) the following viruses and combination of viruses failed to cross infect when first, second, third, or fourth instar larvae were inoculated:

(1) Colias eurytheme Boisduval cytoplasmic polyhedrosis

(2) Pseudaletia unipuncta (Haworth) nuclear polyhedrosis

(3) Pseudaletia unipuncta (Haworth) granulosis

(4) Peridroma (3 samples)

(5) Prodenia ornithogalli Guenée

(6) Pectinophora gossypiella (Saunders)

Combinations of 1+2; 1+3

Pectinophora gossypiella (Saunders) cytoplasmic polyhedral virus has been found to cross infect Trichoplusia ni (Hübner) and Heliothis zea (Boddie) but will not cross infect Galleria mellonella (L.), Ostrinia nubilalis (Hübner), Periplaneta americana (L.), Nauphoeta cinerea (Olivier), Musca domestica (L.). Inoculation of Protoparce sexta (Johannson) with Hyaloicus pinastri cytoplasmic polyhedral virus did not cross infect.

2. Histopathological Investigations. The histopathology of the following insects infected with virus disease has brought to light some startling new information. Sections of infected larvae of the cabbage looper, Trichoplusia ni (Hübner) and the corn earworm, Heliothis zea (Boddie) both showed the formation of polyhedra in the nuclei of the gut cells as well as in the tissues of the hemocoel. This hitherto had not been noted, except by Tanada and mentioned in his studies on the cabbage looper. In effect, this constitutes the recording of a new type of virus disease. Subsequent sections made for the electron microscope disclosed that the cabbage looper

gut nuclear polyhedrosis virus is distinctly different in morphology from the fat body nuclear polyhedrosis. Complete studies have not been made of the corn earworm virus. Cabbage looper virus samples from many localities in the United States were tested by feeding them to larvae and then sectioning the larvae for histopathological study. All samples contain both gut and fat body viruses. Since the cabbage looper virus population is a mixture, bioassay tests and field tests carried out to date have been made with an unknown virus composition which places confidence in the results in jeopardy. We are attempting now to separate the viruses and test them as pure entities. A manuscript on the detection and description of these viruses is now in preparation.

3. Mammalian Toxicology of Nuclear Polyhedral Insect Viruses. A protocol of tests to determine the mammalian toxicology of insect nuclear polyhedral viruses was presented to and approved by the Food and Drug Administration. It includes allergenicity tests on guinea pigs, inhalation tests on white mice, and guinea pigs, intravenous and intracerebral injections in mice, and per os feeding of mice, using the whole polyhedra and isolated particles of insect viruses. A complete series of tests was carried out using the nuclear polyhedrosis virus of the cabbage looper. Approximately 200 animals were included in tests and controls without a single death due to the virus. One mouse died when mauled and bitten by other males in the cage. A manuscript describing this work has been presented for publication and a report will shortly be submitted to the appropriate authorities in the regulatory agencies.

4. Investigations of the Polyhedral Composition from Nuclear Polyhedral Viruses. (a) Detection of Silicon. Common quantitative analysis of silicon (colorimetric), while useful for their intended purposes, are inadequate when used as a means to detect silicon in biological materials, since they are too gross to detect silicon in small samples and suffer from a lack of specificity since the presence of phosphate and/or arsenate ions produce the same color; iron and magnesium were found to depress the color reactions greatly. Modifications of the colorimetric test were devised that eliminate the interference by ions, yielding a method capable of specifically detecting silicon in quantities less than 1 μ Si. Silicon is determined as molybdenum blue by the reduction of a preformed yellow silico-molybdate complex by molybdate ions in the presence of a reducing acid, followed by addition of an alkaline solution. Interference from metallic ions is eliminated by their removal from the system as their insoluble hydroxides. (b) The Silicon Content of the Polyhedra from the Heliothis zea Nuclear Polyhedrosis Virus. The silicon content of the H. zea polyhedra was found to be 0.12% of the dry polyhedral weight. In consideration of nuclear polyhedrosis-virus invasion and infection, the assumption is generally made that the virus rods are liberated from their surrounding polyhedral material within the gut lumen of the host. This liberation is known to involve the dissociation and dissolution of the

polyhedral "protein" material. In the laboratory this liberation is achieved by treatment of polyhedra with dilute sodium carbonate solution (0.003 to 0.04 M) in the presence of sodium chloride (0.05 M) solution. The question remains, however, as to how this process is accomplished in the gut of the animal. That proteolytic enzymes are not involved in the breakdown of the polyhedral "protein" is apparent from two considerations. First, the polyhedral matrix is not affected by the usual proteolytic enzymes, e.g., pepsin, trypsin or papain. Second, if dilute sodium carbonate solution (which is highly incapable of proteolysis) is capable of carrying out the breakdown, there is no reason to think that proteolysis, enzymatic or otherwise, is involved here. Finally, there is direct evidence that protein enzymes of the insect gut are not involved in the breakdown of polyhedral protein to liberate virus rods. As viewed on grids prepared for the electron microscope, whole gut homogenates were seen to be capable of releasing virus rods from whole polyhedra after 1 to 2 hours' incubation. After the same homogenates were heated at 100°C for 20 minutes or deproteinized with 10% trichloroacetic acid, they retained an apparently undiminished ability to release virus rods under the same circumstances.

It is in connection with the breakdown of the polyhedral matrix that the silicon content becomes of interest. Alkaline chloride solutions are notably effective in solubilizing silicates. Thus, if one postulates a three-dimensional framework or skeleton for the polyhedral material about which are "built" the proteinaceous components, another explanation for the dissolution of the polyhedral material becomes feasible. The alkaline chloride solution, as used in the laboratory or as encountered in the gut of the insect, would exert a solvent action upon the silicates of the polyhedron. The proteinaceous components would then become free to pass into solution, or, the in vivo situation, become accessible to the action of gut proteinases. The proteins of the polyhedral matrix are degraded by proteolytic enzymes after alkaline treatment of the polyhedron in vitro. The non-digestibility of these proteins prior to alkaline treatment might in part be simply accounted for on the basis of the hydrophobic properties imparted to the polyhedron by the silicates present. Such a scheme of breakdown shows that after release of the virus rods nearly 80% of the silicon content is in solution and no longer associated with the protein.

In light of this report of silicon in the polyhedral body, the heretofore unexplained significance of magnesium and iron in these bodies becomes amenable to explanation. While a discussion of the structure of silicates will not be attempted, it will suffice to say that the fundamental unit is the orthosilicate ion, SiO_4^{-4}, in which the silicon atom is surrounded tetrahedrally by four oxygen atoms. Each oxygen atom thus bears a net charge of -1, which is balanced by the positive charges of cations packed in the lattice in the interstices of the silicate ions. These positive ions are generally divalent metals, e.g., Fe^{+2} and Mg^{+2}, and, in the most simple consideration, it is they that are responsible for

adjoining the silicate tetrahedra into chains, sheets, etc. of the physical form necessary to constitute a structural element or frame-work. Because of the close correspondnece in the atomic radii of Mg^{+2} and Fe^{+2} ions, these ions, for purposes of this discussion, become interchangeable in the silicate lattice without affecting the structure of the lattice. While such presentation as given here is surely an oversimplification, it nonetheless serves to indicate the importance of magnesium and ferric ions in connection to silicate ions in the formation of the postulated silicaceous frame-work structure of the polyhedral matrix. Finally the detection of silicon has since been supported by quantitative investigations of the silicon content of the same polyhedra material using the absorption spectrophotometer.

5. Nucleic Acid Composition of the Nuclear Polyhedral Virus from Trichoplusia ni (Hübner). Nucleic acid analyses of the nuclear polyhedral bodies of the Trichoplusia ni (Hübner) virus have revealed the presence of both deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Total amounts of DNA and RNA were 12.2+06 and 8.7+09 μg/mg polyhedra, respectively. These findings agree reasonable well with other reports of nucleic acid present in polyhedra from diseases in other insect species.

B. Bacterial Pathogens of Insects

1. Mode of Action of Crystal-Forming Bacteria. Previous investigations of the effect of crystal-forming bacteria on silkworm have shown the silkworm larva is susceptible only to the crystal. Ten minutes after ingestion of the crystal, the gut of the silkworm loses its integrity and begins to leak highly alkaline gut contents into the blood, thus raising the pH of the blood and causing a general total paralysis in from 60 to 90 minutes. Twenty minutes after feeding on crystals, the gut of the silkworm larva becomes paralyzed. Fifty minutes after intoxication, the cellular components of the gut separate and begin to break down.

In an effort to determine the site of action of the crystal on the gut, oral injections of the crystal toxin G-2 along with C^{14}-labeled compounds were administered to last instar larvae of the silkworm B. mori. At predetermined time intervals aliquots of hemolymph were sampled and the radioactivity present was measured. The insects were then sacrificed and fixed for concurrent histopathological examination. Control experiments were performed with the labeled compounds and an innocuous organism B. cereus. The permeability of the intestinal cells C^{14}-labeled carbonate is markedly affected by the presence of crystal toxin, so much so, that differences are noted within the first ten to twenty minutes. More carbonate was observed to enter the hemolymph of toxin treated silkworms than in the controls. The permeability of the intestinal epithelial cells to 1-C^{14}- D glucose was such that transport across the gut cell in the toxin treated silkworms appeared to be inhibited.

When distribution curves of radioactivity vs. time were prepared for the three labeled compounds, only the toxin treated insects in the D-glucose experiment showed a distinct variation from its control, whereas, the carbonate and acetate experimental graphs showed significantly different curves with similar characteristics for both the toxin and control treatments.

The histological examinations showed the characteristic disintegration of the intestinal cells, coinciding with the pathological paralysis and death of the toxin treated silkworm. However, qualitative radioactive differences were detected before histochemical changes were observed, suggesting that the mode of action of toxin first affect cellular and/or permeability functions of the intestinal cells before the massive cell destruction occurs.

2. Isolation of New Strains of Crystalliferous Bacteria. Three new isolates of crystalliferous bacteria have been sent recently to this laboratory from Australia, Riverside, Calif. and Savannah, Ga. The last was isolated by S. R. Dutky from Cadra cantella. It is most interesting that the Australian and California strains were classified as Bacillus thuringiensis var. thuringiensis and they were both a sterotype #7 and vegetative cell esterase type #7, a type found originally in the Orient (Japan) by Aizawa. If one could suppose that the California strain was brought in from the Orient, then the theory of geographic distribution would still be valid. On the other hand, Dutky's organism is a Sero. type 9 or Tolworth type and this originally was isolated and described in England. Again the possibility of transportation across the Atlantic Ocean is not to be excluded.

3. Production of Toxins by Crystalliferous Bacteria on Known Media. Attempts have been made to determine the precise requirements for the production of the toxins by Bacillus thuringiensis, B. thuringiensis var. sotto and Bacillus entoniocidus var. entomocidus on known media. The three organisms were first innoculated into a standard inorganic salt mixture with Na citrate as a source of carbon. Only Bacillus thuringiensis grew under these conditions, and it produced the fly toxin. Combinations of valine, isoleucine and leucine (each at 2.5×10^{-4}M) were added to the basic salt mixture. Neither sotto nor entomocidus produced fly toxin in any of these media. However, thuringiensis produced exotoxin whenever growth took place. The usual LD_{50} of thuringiensis fly toxin was in the range of 0.1-0.2 mg/ml of dried supernatant in sterile water, in the media, to which valine was added. When either leucine or isoleucine, or both were present, growth was inhibited and thus no fly toxin was produced. Addition of valine to the latter media overcomes the negative response.

To date, the density of the solubilization of the crystal toxin from Bacillus thuringiensis as it occurs in the insect gut, is still open to question. It had been suggested that the high pH of the gut in many susceptible Lepidoptera caused dissolution of the crystal by reducing S-S linkages formed during synthesis of the crystalline structure. While such

a hypothesis seemed reasonable, the discovery that the crystal protein contains only 1.1% cystine makes it highly unlikely that the extreme inertness of the crystal is due to S-S linkages since soluble albumen has more cystine than the crystal (1.5% cystine).

Recently it has been discovered that the crystal toxin contains 0.45% of silicon. This is an inordinately high concentration of this element since the media wherein the bacteria grow only contains 0.12% silicon. It is postulated that the silicon plays the same structural role in the crystal as it does in the polyhedron of the nuclear polyhedral virus. This is supported by the similarity in reaction to the environment, and to the insect's gut contents.

Preliminary attempts to grow the crystal-forming bacteria in the absence of silicon from the media have failed. This might mean the silicon is required for growth, but it also impedes the determination of the importance of silicon to crystal formation.

4. Effect of Sunlight on Germination of B. thuringiensis Spores. Clean spores of Bacillus thuringiensis were suspended on millipore filters and exposed to the environment for various times. The amount of light energy and the temperature at which the spore was exposed were recorded and the effects of these were reflected in the percent germination of the spores. Preliminary results indicate that as many as 50 percent of the spores fail to germinate after exposure of 30 minutes in the sunlight.

5. Tests on the Honey Bee (Apis mellifera L.) Colonies with Insect Pathogens. Tests are continuing in cooperation with the Bee Pathology Laboratory on the effects of certain biological insecticides on the honey bee. Various levels of the crystals from different strains of Bacillus thuringiensis were fed to colonies of bees in observation hives. No noticeable effects were observed either on individual workers, the queens, or the entire colonies when crystals were fed at levels ranging from 1.5 million to 12 million crystals per insect.

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AREA NO. 23. INSECT PHYSIOLOGY AND MODE OF ACTION OF INSECTICIDES AND THEIR METABOLITES

Problem. Basic research in insect physiology is essential to the development of more efficient insecticides and new approaches to insect control. The increasing development of resistance to insecticides by insects has emphasized the need for additional information on the mode of action and metabolism of insecticides in insects and the mechanisms of the resistance to insecticides. More knowledge is also needed on the normal physiology and biochemistry of insects to permit a comparison and interpretation of the data obtained from studies on insect toxicology. Basic research in insect biochemistry and physiology, including insect nutrition and metabolism, will provide a better understanding of the biochemical and physiological systems which regulate insect growth, metamorphosis, reproduction, and diapause, and the chemistry and action of the hormones which mediate these systems. Knowledge gained from such research is essential to the development of new methods of effective insect control which are safer and more selective in their action than the methods now being used. More basic information on the response of insects to light, sound, food, and sex attractants could contribute to better insect control. Insects are useful test animals for basic physiological studies on life processes.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term program involving insect physiologists, biologists, geneticists and chemists engaged in basic studies in insect physiology and biochemistry and in the mode of action of insecticides and their metabolites. At the Pioneering Research Laboratory on Insect Physiology at Beltsville, Md., basic research is conducted on biochemistry and physiology of lipids in insects, insect nutrition and hormones, effect of light on insect growth and development, and effect of chemical carcinogens on insects. Research on the effect of light on insect growth and development is also conducted in bioclimatic cabinets at Brownsville, Texas. The Metabolism and Radiation Research Laboratory at Fargo, North Dakota, conducts research on the metabolism of insecticides in insects, biochemistry and physiology of lipids in insects, and insect nutrition and hormones.

The Federal scientific effort devoted to research in this area totals 16.8 professional man-years. Of this number 4.4 is devoted to the biochemistry and physiology of lipids in insects, 4.8 to insect nutrition and hormones, 1.4 to effect of light on insect growth and development, 0.2 to effect of chemical carcinogens on insects, and 6.0 to metabolism of insecticides in insects.

In addition the Federal support of research under contract with the Mississippi State University provides 0.5 man-year devoted to insecticide resistance in vertebrates.

Additional research in this area is provided by the following P. L. 480 projects: S5-ENT-3 Colombia (2 professional man-years); A7-ENT-6 India (2 professional man-years); A7-ENT-14 India (2 professional man-years); E21-ENT-3 Poland (1 professional man-year); and E21-ENT-4 Poland (1 professional man-year).

PROGRAM OF STATE EXPERIMENT STATIONS

The State stations have an extensive research program in insect physiology. Respiration and the intermediate metabolism of nitrogen compounds; the excretion of nitrogen in the insect; the feeding behavior and nutrition of plant sucking insects, especially aphids; the relationship between form and function in insect blood cells; the metabolism of amino acids, phospholipids and other nutrients in insects; membrane barriers in insects and the effects of temperature on them; position and chemical structure of lipid solvent extractive components of the exoskeleton of the Mormon cricket; effect of visible spectrum irradiation on growth of insects; special glands of insects that produce defense substances used for protection against predators and against systemic infection by microorganisms; location, structure and function of chemoreceptors; biochemical changes in insects as a result of development and aging; effects of X-radiation on the embryos of insects; response of insects of economic importance to radiant energy; certain Krebs cycle reactions and respiratory control in isolated flight muscle sarcosomes; major catabolic pathways for purine and pyrimidine nucleotides in flight muscles of insects; and culture methods for mandibulate and sucking plant feeding insects are being studied. Selected insects are reared on artificial diet as a means of determining their physiological and biochemical characteristics.

Mode of action of insecticides is being studied at a number of State stations. The metabolism of various insecticides in insects is studied using paper chromatography, radioactive tracers and other techniques. The effect of the size, shape and molecular configuration of insecticides and related molecules to their toxic action, the biochemical action of insecticides and drugs on enzyme systems of the insect body and the detoxification of toxic molecules in insects are being studied. Special emphasis is on organophosphorus and carbamate toxicants in much of this research. Insect resistance to insecticides is being studied with the ultimate objective of developing effective and lasting chemical control measures. The metabolic fate of certain organophosphorus insecticides after ingestion by poultry is being determined. Systematic investigations are being made of the in vitro metabolism of organophosphate insecticides by a number of tissues from several insect species. Known metabolites of insecticides are tested for their effects on the metabolic reactions of the insects. Rates and routes of entry and distribution of insecticidal chemicals in insects and intoxication, detoxication, tolerance and excretion mechanisms are being studied. The effect of population density, temperature, light and other environmental factors on toxic action of insecticides is being determined. A biological study is being made to determine the changes that occur in populations of

boll weevils that influence their resistance to insecticides. Chemicals which will inhibit the detoxifying enzymes in resistant insects and etiological mechanisms which enable insects to tolerate unusual amounts of insecticides are being studied. The action of ovicides on insect and mite eggs is being determined. Physiological effects of insecticides including synergism, antagonism and other factors are being investigated.

The effect of stresses (chemical insecticides, electric shock, thermal stress, specific enzyme inhibitors) on the elaboration of neurotoxins and on central nervous system activity is being determined. The metabolic fate of insecticides in plants is being determined. A regional project has been developed in this area. Genetic studies are being made of the resistance of insects to insecticides.

Chemical attractants, both feeding and sex stimulants, for insect pests are being studied and the use of these attractants for insect control in baits, sprays and traps is being investigated.

There are 48.4 professional man-years devoted to this area at the State stations.

PROGRESS --- USDA AND COOPERATIVE PROGRAM

A. Biochemistry and Physiology of Lipids in Insects

1. Identification of the major constituents of the crystalline powder covering the larval cuticle of Samia cynthia ricini. In studies at Beltsville a white crystalline powder was observed to accumulate superficially on the larval cuticle of Samia cynthia ricini. The crude powder (m.p. 86°C) collected by brushing it from the larvae with a camel's hair brush, was shown to be primarily saturated alcohols by infrared spectroscopy, thin-layer (TLC), and gas-liquid chromatography (GLC), and through the formation of several derivatives. Although the major components of the crude powder migrated on thin layers of silica gel in a manner identical with straight chain saturated alcohols, trace quantities of hydrocarbons, esters, sterols, and acids were also detected. After chromatography on a Florisil column, the alcohol fraction (92.6% of the mass) was found by TLC to be free of contamination. Analysis by GLC of this alcohol fraction revealed a minor and a major peak. Comparison of the retention times of these compounds with a log plot of known standard saturated straight chain alcohols indicated that the two peaks were n-octacosanol (0.6%) and n-triacontanol (99.4%).

Oxidation of the cuticular alcohol with chromic acid gave triacontanoic acid and the corresponding hydrocarbon (n-triacontane) was prepared from the alcohol via tosylation and reduction with lithium aluminum hydride. The physical properties and relative retention times of these derivatives further confirmed the structure of the cuticular alcohol. The absence of branching was shown by the use of molecular sieves.

To determine whether these alcohols are biosynthesized by the insect or derived from the host plant, radiotracer studies were undertaken using the 1-C^{14}-acetate. Radioassay of the cuticular lipids 24 hours after the injection of C^{14}-acetate showed about 0.75% incorporation of this labeled precursor. About 96% of the total C^{14} compound present in the cuticular lipids was eluted in the alcohol fraction following chromatography on Florisil. GLC analysis of this crystalline material gave the two peaks corresponding to n-triacontanol and n-octacosanol. Trapping of the effluent from GLC column showed 85.7% of the recovered activity to be present in the n-triacontanol peak, thus demonstrating endogenous biosynthesis.

These cuticular alcohols do not form a contiguous sheet or coating as the "waxes" of many other insects, but rather exist on the larval cuticle as a fine powder. Since the alcohols compose so much of the cuticular lipid, it is tempting to speculate about their possible involvement in water conservation, a role traditionally applied to cuticular lipids. However, the noncontiguity of these alcohols mitigates this view.

2. Improved synthesis of brassicasterol and 22,23-dihydrobrassicasterol. For comparative studies on the utilization and metabolism of campesterol and 22,23-dihydrobrassicasterol (C-24 methyl isomeric sterols) by the house fly and other insects, the availability of gram quantities of 22,23-dihydrobrassicasterol was required. Since the method most commonly employed for the preparation of brassicasterol and 22,23-dihydrobrassicasterol gives the first intermediate in about 21% yield and the brassicasterol acetate in an overall 4% to 5% yield, an alternate method for the preparation of 22,23-dihydrobrassicasterol was investigated at Beltsville.

The solvolysis of ergosterol tosylate followed by chromatography on alumina gave i-ergosterol in 55% yield. From the oxidation of i-ergosterol with chromic acid in pyridine, 3,5-cycloergosta-7,22-diene-6-one (III) was obtained in 70% yield. The reduction of III with lithium and liquid ammonia yielded 3,5-cycloergosta-22-ene-6-one (IV). Lithium aluminum hydride reduction of IV afforded 3,5-cycloergosta-22-ene-6α-ol (V) and the catalytic hydrogenation of V gave 3,5-cycloergosta-6β-ol (VI). The rearrangement of VI with zinc acetate in boiling acetic acid, followed by the saponification of the resulting acetate, gave 22,23-dihydrobrassicasterol. Rearrangement of V under similar conditions yielded brassicasterol.

Brassicasterol and 22,23-dihydrobrassicasterol were obtained in an overall yield of 25% and 20%, respectively. This method of preparation also eliminated the high temperature sealed tube reaction that was used in previous syntheses.

3. Conversion of Cholesterol to 7-Dehydrocholesterol in Aseptically Reared German Cockroaches. The conversion of cholesterol to 7-dehydrocholesterol by nonaseptically reared German cockroaches has been confirmed using aseptically reared insects. The identity of the 7-dehydrocholesterol was established by ultraviolet and infrared spectroscopy and by gas-liquid

chromatographic analyses of the free sterols and/or derivatives. This metabolic conversion was previously found to occur in certain other insects, including the house fly and the American cockroach. Nutritional studies at Beltsville have also shown that 7-dehydrocholesterol will fulfill the "essential sterol" requirement of aseptically reared house fly larvae when provided jointly with a "sparing sterol." In addition, a high concentration of 7-dehydrocholesterol has been found in the prothoracic gland of the American cockroach during the terminal nymphal molt. These findings and the recent report that the molting hormone (ecdysone) is a Δ^7, 6 keto steroid suggest that 7-dehydrocholesterol is an intermediate in the conversion of cholesterol to a steroid hormone(s) in insects.

B. Insect Nutrition and Hormones

1. Feeding Stimulants For the Female House Fly. At Beltsville yeast and casein hydrolysates were found to contain feeding stimulants which caused the female house flies to aggregate in clusters and feed avidly and to extend their probosces when their tarsi were stimulated with the materials. Guanosine monophosphate was isolated in pure form and identified as the major active component in the yeast hydrolysate. Several amino acids, including leucine, methionine, lysine, and isoleucine, were found to be the active components in the casein hydrolysate. The more potent of these stimulants, when impregnated on filter paper, will cause female flies to cluster and feed at microgram levels and will bring about proboscis extension in about 50% of the insects at concentrations as low as 0.005 M. The presence of the phosphate ion with the feeding stimulant was found to be essential for activity for either clustering and feeding or tarsal stimulation. The relative response of the female fly to these compounds was also dependent both upon the age of the fly and the state of ovarian development. These feeding stimulants, which are important tools for studying insect behavior and feeding, may have practical applications as components of pesticide-bait combinations.

2. The Uptake of Feeding Stimulants by the House Fly. A study was made at Beltsville on the uptake by female house flies of solutions of feeding stimulants either alone, in combination, or in competition with solutions of certain other nutrients. The uptake of solution was measured daily for groups of female flies for a period of 10 days. The solutions, tested in 2-choice or 3-choice situations, included 2.5% albumen, 0.5 M sucrose, 0.05 M l-leucine, and 0.02% guanosine-5' monophosphate (GMP-5'). All the materials were made up in a 0.133 molar phosphate buffer except sucrose which was made up in distilled water.

The uptake of the solutions of the two feeding stimulants, l-leucine and GMP-5', alone was equal to or slightly higher than the uptake of sucrose solution. With each of these stimulants there was an initial high uptake during the first four days, with a gradual decrease during the remaining six days. When the two feeding stimulants were combined with egg albumen,

the uptake was greatly synergized with the flies ingesting three times as much l-leucine-albumen and five and one-half times as much GTP-5'--albumen solutions as sucrose solution. The uptake of the albumen alone does not occur until the fourth day after emergence and the volume of albumen solution ingested is only about one-half that of sucrose. There was no increase in uptake when the feeding stimulants were used in combination with sucrose. It is concluded that these feeding stimulants synergize the uptake of protein and further that the uptake of these compounds is related to certain physiological requirements brought about by the ovarian cycle of the female fly.

3. Selection for Nutritional Autogeny in the House Fly. It was previously shown that when house flies were reared on a larval diet containing cholesterol that about half of the adult female flies were autogenous, i.e., these flies developed mature ovaries and produced eggs on a diet consisting solely of sucrose and water in the absence of any exogenous source of protein. Increasing the concentration of cholesterol in the larval diet did not result in an increase in the percentage of females developing mature ovaries. This suggested that perhaps the population of flies under study was a mixture of autogenous and anautogenous flies and that perhaps, as in the case of certain species of mosquitoes, an homogenous strain of autogenous house flies could be selected. By repeatedly rearing larvae at Beltsville in CSMA medium with added cholesterol, and collecting eggs from emerging adults held on a diet of sucrose and water only, selection toward autogeny would be accomplished since progeny would be available only from females capable of ovarian development under these conditions. To test the procedure the following method was employed: Flies from a 1948 NAIDM strain were reared on CSMA medium containing 1% cholesterol. Pupae were separated from the medium and adults permitted to emerge in a clean cage and held on a diet of sucrose and water. Eggs were collected from the female flies fed only on sucrose and water and the selection process was carried through a number of succeeding generations. Six days after total emergence approximately 100 female flies from each generation were examined for ovarian development. This selection has now been carried through 60 generations and the percentage of females from this strain that now develop mature ovaries when fed on a larval diet containing cholestrol and an adult diet of sucrose and water is between 90% and 99%. An attempt is now underway to select an anautogenous strain of flies from the parent stock and these two strains will be compared biochemically to study the role of cholesterol in the initiation of ovarian development in the adult house fly.

4. Isolation and Identification of Insect Neurohumoral Substances. As neuromuscular function in insects does not seem to depend on a cholinergic system, a better understanding of the chemical mediator and the associated enzyme systems should provide a basis for the development of selective control measures. A substance previously reported to stimulate muscular activity in crustacea was investigated for possible effects in insects at Fargo. Several column chromatographic procedures were used to isolate a substance from extracts of three insects: a cockroach, the house fly, and

the false wireworm. This material was bioassayed by applying it to isolated thoracic nerve cords of P. americana. The spontaneous efferent activity was first increased, then was followed by complete blockage of activity.

Further chemical characterization of the unknown seemed to indicate that it may be carnitine or a derivative of carnitine. Carnitine has been found in moderate concentrations in nervous tissues of P. americana. The presence of biogenic amines in several species of insects is being investigated. Five catecholamines have been isolated from the house fly, the cabbage looper, and the cockroach. The chemical identification of these catecholamines is continuing.

C. Effect of Light on Insect Growth and Development

1. Sensitivity of Visual Receptors of Carotenoid-Depleted Flies. Adult house flies raised under sterile conditions on a larval diet lacking carotenoids and retinol (vitamin A) have visual receptor sensitivities--as assessed electroretinographically--which average more than 2 log units below normal, both in the near ultraviolet (340 mμ) and visible (500 mμ) regions of the spectrum. Loss of sensitivity can be prevented by the addition of β-carotene to the larval food. At Beltsville, flies reared for several generations on a carotenoid-free diet, but under conditions where the adults are not kept sterile, do not show a further loss of sensitivity. It is suggested that carotenoid stored in the egg prevents complete blindness in the first generation, and that microorganisms can supply small amounts of carotenoid to the adults and thereby prevent complete blindness in the second and successive generations. This is the first definite demonstration of a physiological role for carotenoids in insect vision. (This research was conducted in cooperation with Yale University.)

2. Effect of Meteorological Factors on Insect Diapause. At Beltsville studies were initiated to explore the possibilities of controlling harmful insect populations by manipulating photoperiods. Research now underway includes investigation of a number of aspects of the action of light on insects, such as measurement of light penetration into fruits, vegetables, cotton bolls and soil and determination of the spectral characteristics of the light most effective in controlling diapause in insects. Studies were initiated in the bioclimatic cabinets at Brownsville on the effect of photoperiods, temperature and relative humidity on diapause of selected insect species. Exploratory studies were undertaken with tobacco budworm larvae. Cotton bollworm and pink bollworm will be included when reared stocks become available.

D. Metabolism of Insecticides in Insects

1. Isolation and Characterization of Insect Esterases and Phosphatases. A more sensitive method was developed at Fargo for the detection of esterases in cockroach homogenates. By means of column chromatography,

five major esterase fractions were obtained. A technique was developed for purification of an esterase found in the gut and hemolymph using acetone precipitation, ammonium sulfate fractionation and heat treatment. Two of the esterases located in the alimentary tract were not inhibited by 10^{-4} M DDVP. Three different phosphatases were isolated from the gastric secretion of cockroaches. Efforts are now underway to further characterize the various esterases and phosphatases with suitable substrates and inhibitors.

E. General

1. Brain and Liver Sterols of Young Rats Fed 20,25-Diazocholesterol. When the hypocholesterolemic agent, 20,25-diazocholesterol, was fed to weanling rats at 0.03% in a cholesterol deficient diet for 3 and 6 weeks, both the brain and liver sterols were found to contain large concentrations of desmosterol, whereas only cholesterol was detected in the brain sterols of control rats. To determine the origin of the brain desmosterol, rats that had been held on a diet containing the diazosterol for 3 or 6 weeks were injected with either C^{14}-acetate or C^{14}-glucose. The brain desmosterol was isolated and found to be labeled. A comparison of the rates of incorporation and specific activities of the sterols suggests that the desmosterol present in the brain is synthesized in the brain rather than transferred from the serum. This indicates that this hypocholesterolemic agent may directly affect normal brain sterol metabolism. (This research was conducted in cooperation with the Human Nutrition Research Division, ARS.)

2. Effect of Gravity on Insect Development. At Beltsville Aedes aegypti mosquito eggs were subjected in an ultracentrifuge to forces of 150,000 to 198,000 times the normal earth's gravity. A high percentage of the mosquitoes hatched and survived to the adult stage, indicating that the mosquito eggs can withstand enormous stress.

3. Effect of Circadian Rhythm on Sensitivity of Insects to Insecticides. At Beltsville a study is in progress to establish whether there is a circadian rhythm of variation in sensitivity of insects to insecticides. Tests are being conducted around the clock with pyrethrins against house flies, cockroaches, and Japanese beetles.

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AREA NO. 24. FUNDAMENTALS OF INSECT STERILITY

Problem. Basic research on insect sterility is needed to determine if this new approach can be used to control or eradicate destructive insects, thus eliminating the hazards often associated with the application of insecticides to crops and livestock or the high cost of other insect-control measures. The sterile-male technique, involving the use of gamma radiation to produce the sterility, and the release of dominant numbers of laboratory-reared sterilized males, has been utilized successfully to eliminate the screw-worm from the Southeast. The use of a sterilizing chemical in combination with a bait to attract insects already in the environment has tremendous possibilities and may prove more widely useful than radiation sterilization, because insects need not be reared in the laboratory to outnumber native insects. If a majority of native insects can be attracted and sterilized, thus outnumbering the remaining insects in the population, the same effect may be achieved without the expense of rearing, sterilizing, and releasing sterile males. This field is not necessarily limited to the use of baits containing sterilizing chemicals which insects will eat. The insects might be attracted to a light or an odor and receive a sterilizing dose of chemical through contact. Other approaches include the production of mutations in laboratory strains of insects which would not be lethal in the laboratory but would be lethal in nature. Much additional basic work is needed on the genetics and physiology of reproduction of insect pests, and on the effects of various types of sterilants, in order to determine the possibilities inherent in these new approaches to insect control and whether or not they could be utilized to destroy the many insects of economic importance.

USDA AND COOPERATIVE PROGRAM

The Department has a continuing long-term research program on insect sterility and its application to control and eradication of insect pests. Basic research on the fundamentals of insect sterility is conducted at the Metabolism and Radiation Research Laboratory established at Fargo, North Dakota, in October 1963. The research is in cooperation with Crops Research and Animal Husbandry Research Divisions and with the North Dakota Experiment Station. Research on sterility in insects produced by gamma radiation and chemosterilants directed principally toward practical application to control specific insects is also conducted at a number of field laboratories and is discussed under other areas.

The Federal scientific effort devoted to research in this area totals 7.0 professional man-years. Of this total 3.5 is devoted to basic studies on radiation sterilization, 1.5 to effects of mutagenic chemicals on reproduction and heredity, 1.0 to cellular effects from exposure to chemical mutagens or radiation, and 1.0 to genetics of selected economically important insects.

In addition Federal support of research in this area under a grant provides for 0.5 man-years devoted to effect of radiation on insect behavior and tropisms.

PROGRAM OF STATE EXPERIMENT STATIONS

Research on the fundamentals of insect sterility at the State stations includes the physiological and toxicological effects of chemosterilants on insect metabolism and reproduction. Radio-labelled sterilants and their biochemical intermediates are being used in vivo and in vitro studies to determine the influence of these compounds on amino acids, nucleic acids, proteins and enzymes in insects. Research is also being performed to determine the cytological and histochemical effects of irradiation and chemosterilants on insects in varying stages of development and maturation. Studies designed to determine dosages required for sterilization and methods of field application are included in this report under the particular commodity on which the research is being conducted.

A total of 2.0 professional man-years is devoted to studies on the fundamentals of insect sterility at the State stations.

PROGRESS--USDA AND COOPERATIVE PROGRAM

A. Basic studies on radiation sterilization.

1. Boll weevil. A colony of the Texas strain of boll weevil has been established at Fargo and is producing satisfactorily. The weevil strain from the Boll Weevil Laboratory at State College, Mississippi, has been discontinued due to relatively poor reproductive performance of the colony at the Fargo laboratory. The larval and adult artificial diets from the Boll Weevil Laboratory recipe have been adopted as standard.

Studies were conducted on irradiation of the head and anterior thorax, abdomen and posterior thorax, and the whole body. The results showed that the sensitivity of the boll weevil to irradiation is due to damage which occurs in the abdomen and posterior part of the thorax. Irradiation of the head and anterior thorax had no effect on egg production or fertility. Irradiation with 7,500 r produced no mortality of weevils in the succeeding 21 days when the head and anterior thorax only was exposed. Mortality was 95 and 100 percent respectively when abdomen and posterior thorax and the whole body was exposed.

A dosage of 5,000 r of gamma rays was more lethal when applied to 4-day old weevils at a rate of 600 r/min than when the application rate was 10 r/min. Application of 7,500 r in three fractions of 2,500 r each delivered at intervals ranging from a few hours to 24 hours apart showed no difference in the effect over the application of the total dosage of 7,500 r in a single exposure.

House fly. Male house flies were exposed to 1000 r X-rays at 100 and 1000 r/min and 1000 r gamma rays at 100 and 10,000 r/min. The number of dominant lethals produced was not significantly different for either type or rate of radiation. This indicates that employing low rates to overcome somatic damage should have no appreciable effect on dominant lethal production required to produce sterility.

B. Effects of mutagenic chemicals on reproduction and heredity.

Long exposure of Habrobracon males to tarsal contact with residual films of tretamine and hempa resulted in some mortality within 24 hours. Fairly brief exposures permitted adequate survival to permit tests of the sperm for dominant lethal mutations or sperm inactivation. Preliminary results indicate that some degree of sperm inactivation and a high rate of dominant lethal mutations are effected.

C. Cellular effects from exposure to chemical mutagens or radiation.

Adult boll weevils were irradiated with 2,000, 5,000, and 8,000 r of X-rays and the midguts were subsequently examined to determine the effect. At 9 days after irradiation all regenerative cells and much of the secretory epithelium were absent in the midguts of those receiving 5,000 and 8,000 r. The midguts of those receiving 2,000 r appeared to be essentially normal.

D. Genetics of economically important insects. Larval ganglion cells of various stocks of house flies have been examined for morphological chromosome characteristics. The karotypes, including differences, have been worked out for a few stocks. Using the Orlando stock as a reference strain (normal - with respect to the five pairs of autosomes), the following stocks have been found to possess a normal karotype: Rl/+; cm; ge, bwb; and Lp/+.

PUBLICATIONS--USDA AND COOPERATIVE PROGRAM

Basic Studies on Radiation Sterilization.

Flint, Hollis M. 1965. The Effects of Gamma Radiation on the Fertility and Longevity of Hippelates pusio. J. Econ. Entomol. 58: 555-9.

LaChance, L. E. and M. M. Crystal. 1965. Induction of Dominant Lethal Mutations in Insect Oocytes and Sperm by Gamma Rays and an Alkylating Agent: Dose-Response and Joint Action Studies. Genetics 51: 699-708.

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ENT b1(R)	Sugarbeet insect investigations			
ENT b1-1 (R)	Control methods and biological studies of insects and mites affecting sugarbeets	Mesa, Ariz.	Yes	11-G-1
		Logan, Utah	Yes	11-B-1
		Twin Falls, Idaho	Yes	11-A-1
				11-B-1
		Yakima, Wash.	Yes	11-A-1
				11-B-1
				11-C-1
				11-F-1
				11-G-1
ENT b2(R)	Tobacco insect investigations			
ENT b2-1 (R)	Biological control methods and biology of insects attacking tobacco foliage	Oxford, N.C.	Yes	10-A
				10-D
		Florence, S.C.	Yes	10-A
		Quincy, Fla.	Yes	10-A
				10-D
ENT b2-2 (R)	Insecticide control methods for insects attacking tobacco foliage	Oxford, N.C.	Yes	10-C
		Quincy, Fla.	Yes	10-B
				10-C
		Florence, S.C.	Yes	10-B
ENT b2-3 (R)	Control methods and biology of soil insects that attack tobacco	Florence, S. C.	Yes	10-B
ENT b2-4 (C)	Attractants, hormones, and sterilization procedures for control of tobacco insects	Lexington, Ky.	No	
		Oxford, N.C.	Yes	10-E
		Raleigh, N.C.	No	
		Florence, S.C.	Yes	10-E
		Clemson, S.C.	No	
		Quincy, Fla.	Yes	10-E
		Blacksburg, Va.	No	
		St. Croix, Virgin Islands	Yes	10-A
				10-E
ENT b2-5 1/ (Gr)	Basic studies on the nature and significance of weather as a tool for the prediction and behavior of field populations of insects	Clemson, S.C.	No	
ENT b3(R)	Greenhouse and ornamental plant insects			
ENT b3-1 (R)	Biology and methods of control of insects on greenhouse and ornamental plants	Farmingdale, N.Y.	Yes	12C-1
				12-E-4,6
				12-F-1
				12-G-2
		Beltsville, Md.	Yes	12-A-4
				12-B-1,2, 3,6
				12-D-1
				12-E-5
				12-F-1
		Sumner, Wash.	Yes	12-A-1
				12-B-5
				12-E-1
				12-G-1
ENT b4	Vegetable and berry insects			
ENT b4-1 (R2)	Biology and methods of control of insects and mites affecting beans and peas	Twin Falls, Idaho	Yes	1-B-2
		Beltsville, Md.	Yes	1-C-1,3
		Yakima, Wash.	Yes	1-H-1
ENT b4-3 (R)	Biology and methods of control of insects affecting melons and other cucurbits	Beltsville, Md.	Yes	1-C-1
				1-E-5
		Charleston, S.C.	No	
ENT b4-4 (R)	Biology and methods of control of the beet leafhopper as a pest of vegetables	Twin Falls, Idaho	Yes	1-B-2
		Logan, Utah	Yes	1-B-2
ENT b4-5 (R)	Insects in relation to diseases of vegetables and berries	Yakima, Wash.	Yes	1-H-1
				2-G-1
		Orono, Me.	Yes	2-G-2

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ENT b4-6 (R2)	Biology, host plant relationships, and methods of control of insects that attack potato	Orono, Me.	Yes	2-A-1 2-B-1 2-D-1
		Yakima, Wash.	Yes	2-A-1,2,3 2-B-1,2,3 2-E-1 2-F-1,2
ENT b4-7 (R)	Methods of preventing deleterious residues resulting from the use of insecticides on vegetables and berries	Charleston, S.C.	Yes	1-C-4 2-A-4 2-F-3
		Yakima, Wash.	Yes	2-C-2,3,4
		Beltsville, Md.	Yes	1-B-5 1-C-1,2,3,6 2-C-2
		Orono, Me.	Yes	2-C-1,2
ENT b4-8 (R)	Investigations on the use of natural enemies and other biological methods for the control of vegetable and berry insects	Twin Falls, Idaho	Yes	1-D-5
		Riverside, Calif.	Yes	1-A-1,2,3,4,5,6 1-D-3 1-E-3
		Beltsville, Md.	Yes	1-E-4,5 3-D-3
		Charleston, S.C.	Yes	1-D-2,3 1-E-1,2 1-G-2
		Yakima, Wash.	Yes	1-D-3,4
		Mesa, Ariz.	Yes	1-A-7,8 1-D-3
ENT b4-9 (R)	Biology and methods of control of insects and mites affecting strawberries and bramble berries	Beltsville, Md.	Yes	3-D-3
ENT b4-10 (R2)(C)	Biology and methods of control of insects affecting underground portions of vegetables	Charleston, S.C.	Yes	1-A-9,10,11 1-B-4 1-G-1
		Baton Rouge, La.	Yes	1-A-11 1-B-4 1-G-1
		Yakima, Wash.	Yes	1-B-3
ENT b4-12 (R)	Improvement of methods and evaluation of equipment for applying insecticides to vegetable crops	Forest Grove, Oreg.	Yes	1-F-4,5,6
		Beltsville, Md.	Yes	1-F-3
		Charleston, S.C.	Yes	1-F-1
ENT b4-14 (R2)	Development of methods for preventing contamination of processed vegetables and berries by field insects	Beltsville, Md.	Yes	1-B-5 1-E-4
ENT b4-16 1/	Control of insects and mites affecting vegetable and berry crops through the development of resistant plant varieties	Beltsville, Md.	No	
		Charleston, S.C.	No	
		Riverside, Calif.	No	
ENT b4-17 1/ (CA)	Biological control of aphids attacking potatoes	Orono, Me.	No	
AE-ENT-2 1/ (Gr)	The influence of electromagnetic energy on green peach aphid Myzus persicae (Sulzer)	Lafayette, Ind.	No	
CR-ENT-3 1/ (Gr)	Basic research on insect host-plant interactions involved in plant resistance to the potato leafhopper	Ames, Iowa	No	
ENT-0-0-4 1/ (AID)	Biology, ecology, and development of methods for the control of insect pests of beans, peas, and other vegetable legumes in Asia	Karaj, Iran	Yes	1-A-13
ENT b5(R)	Methods of treating plants and commodities regulated by plant quarantines			

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ENT b5-1 (R)	Development of treatments for plants and commodities regulated by plant quarantines	Hoboken, N.J.	Yes	4-G-3
ENT b6	Mexican fruit fly and other fruit pests in Mexico that threaten U. S. horticulture			
ENT b6-1 (R)	Biology, ecology, and methods for control of the Mexican fruit fly and citrus blackfly	Mexico City, Mex.	Yes	4-A-2 4-E-2
ENT b6-2 (R)	Studies of lures for Mexican fruit flies	Mexico City, Mex.	No	
ENT b6-4 (R)	Quarantine treatments for Mexican fruit fly infested fruits	Mexico City, Mex.	No	
ENT b7	Investigations of fruit flies in Hawaii			
ENT b7-1 (R)	Ecology and biology of fruit flies and their natural enemies in Hawaii	Honolulu, Hawaii Hilo, Hawaii	Yes No	4-A-2
ENT b7-2 (R)	Development of new or improved mass production methods and manipulation techniques for fruit flies and their biological control agents	Honolulu, Hawaii	No	
ENT b7-3 (R)	Investigation of fruit fly lures and repellents in Hawaii	Honolulu, Hawaii Hilo, Hawaii	Yes No	4-A-2
ENT b7-8	Development of methods for eradication and control of fruit flies in Hawaii	Honolulu, Hawaii Hilo, Hawaii	Yes No	4-A-2 4-E-2 4-F-1
ENT b7-9	Commodity treatments to destroy fruit flies and associated pests of quarantine importance in fresh fruits and vegetables in Hawaii	Honolulu, Hawaii	Yes	4-G-1
ENT-0-0-3 (AID)	Development of sterilization procedures for the eradication and control of the Mediterranean fruit fly	San Jose, Costa Rica	Yes	4-A-2
ENT b8	Deciduous fruit and nut insect investigations			
ENT b8-1 (R)	Studies of the codling moth and its control	Yakima, Wash. Wenatchee, Wash. Kearneysville, W. Va. Vincennes, Ind.	Yes Yes Yes Yes	3-A-1 3-B-1 3-E-1 3-E-1 3-B-1 3-A-1 3-B-1 3-E-1
ENT b8-2 (R)	Studies of orchard mites and their control	Yakima, Wash. Wenatchee, Wash. Kearneysville, W. Va. Vincennes, Ind.	Yes Yes Yes Yes	3-B-2 3-B-2 3-B-2 3-B-2
ENT b8-3 (R)	Studies of the plum curculio and its control	Ft. Valley, Ga. Vincennes, Ind.	Yes No	3-A-3 3-B-5
ENT b8-4 (R)	Studies of borers attacking deciduous fruit trees and their control	Ft. Valley, Ga. Vincennes, Ind. Kearneysville, W. Va.	Yes Yes Yes	3-A-2 3-A-2 3-E-2 3-B-5
ENT b8-6 (R)	Studies of miscellaneous insect and mite pests of deciduous fruits and their control	Ft. Valley, Ga. Vincennes, Ind. Yakima, Wash. Wooster, Ohio Wenatchee, Wash.	No Yes No Yes Yes	3-B-5 3-E-3 3-A-3 3-B-5 3-E-3 3-B-5 3-E-3

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ENT b8-7 (R)	Investigations of nut insects and mites and their control	Albany, Ga.	Yes	3-A-4 3-B-3 3-E-3
		Shreveport, La.	Yes	3-A-4 3-F-1
		Wooster, Ohio	Yes	3-B-5 3-E-3
ENT b8-8 (R)	Grape insect investigations	Wooster, Ohio	Yes	3-B-5
ENT b8-10 *	Ecology, biology, and control of the pear psylla	Yakima, Wash.	No	
		Wenatchee, Wash.	Yes	3-E-3
ENT b8-11 1/ (Gr)	Basic studies on the influence and significance of photoperiod and light on diapause and development of the codling moth	Pullman, Wash.	No	
ENT b8-12 1/ (Gr)	Basic studies on the nature and significance of chemosterilant and attractant techniques for eradication of the oriental fruit moth	Grand Junction, Colo.	No	
ENT b8-13 1/ (Gr)	Mass rearing and biology of the peach tree borer (Sanninoidea exitiosa (Say))	Raleigh, N.C.	No	
ENT b8-14 1/ (Gr)	Basic studies on the nature and significance of sex pheromones and gamma radiation induced sterility of the navel orangeworm, Paramyelois transitella (Walker)	Berkeley, Calif.	No	
ENT b8-16 1/ (Gr)	Ecology of mites within pomaceous tree fruit orchards	Provo, Utah	No	
ENT b8-17 1/ (Gr)	Basic studies on the behavior of the pear psylla (Psylla pyricola (Förster))	Wenatchee, Wash.	No	
ENT b9(R)	Investigations of insect and mite vectors of deciduous tree fruit viruses			
ENT b9-1 (R)	Distribution of insects and mites in and near deciduous fruit orchards infected with virus diseases	Riverside, Calif.	Yes	3-A-5
ENT b9-2	Studies of insect vectors of phony peach virus disease and their control	Ft. Valley, Ga.	Yes	3-B-4 3-G-2
ENT b9-3 (R)	Studies of mite vectors of peach mosaic virus disease, including biology, ecology, and control	Riverside, Calif.	Yes	3-A-5 3-B-4
ENT b9-4 (R)	Transmission studies with possible insect and mite vectors of the latent group of stone fruit viruses	Corvallis, Oreg.	Yes	3-G-2
ENT b9-8	Studies of possible insect and mite vectors of pear decline and their control	Riverside, Calif.	Yes	3-A-5 3-G-1
ENT b9-9	Transmission studies with possible insect and mite vectors of miscellaneous viruses causing diseases of deciduous fruits	Ft. Valley, Ga.	No	
		Wenatchee, Wash.	No	
		Corvallis, Oreg.	Yes	3-A-5
ENT b10	Insects of citrus and other subtropical fruits			
ENT b10-1 (R)	Biology and methods of control of citrus mites	Orlando, Fla.	Yes	4-A-1 4-B-1
		Riverside, Calif.	Yes	4-A-1
ENT b10-2 (R)	Biology and methods of control of scale insects, whiteflies, and mealybugs on citrus	Orlando, Fla.	Yes	4-H-1
		Riverside, Calif.	Yes	4-A-1 4-B-1 4-E-1
		Weslaco, Tex.	Yes	4-A-1 4-C-1
ENT b10-3 (R)	Biology and methods of control of miscellaneous insects on citrus and other subtropical fruits	Riverside, Calif.	No	
		Honolulu, Hawaii	Yes	4-A-3
		Mexico City, Mex.	No	
ENT b10-4 (R)	Insect vectors of tristeza and other diseases of citrus	Orlando, Fla.	No	
ENT b10-5 (R)	Investigations of the biological control of citrus insects and mites	Orlando, Fla.	Yes	4-D-1
		Riverside, Calif.	Yes	4-D-1
		Weslaco, Tex.	Yes	4-D-1

* Supersedes ENT b8-5

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ENT b11	Japanese beetle, European chafer, and related species			
ENT b11-1 (R)	Investigations of methods for controlling the Japanese beetle and eradicating isolated infestations	Moorestown, N.J.	Yes	12-B-4 12-E-2
ENT b11-2 (R)	Development and improvement of treatments to permit movement of nursery stock and farm products under quarantine regulations	Moorestown, N.J.	No	
ENT b11-3 (R)	Development of methods of making biological assays of insecticidal residues in soils	Moorestown, N.J.	No	
ENT b11-4 (R2)	Investigations of survey methods and biological and chemical control of the European chafer	Geneva, N.Y.	Yes	12-A-3 12-E-3
ENT b11-6	Ecology, biology, and natural control of the Japanese beetle	Moorestown, N.J.	Yes	12-A-2
ENT b11-7 1/ (Gr)	Basic studies of the biology of the Cuban may beetle	Gainesville, Fla.	No	
ENT c1	Boll weevil investigations			
ENT c1-1 (R)	Biological research on the boll weevil	Florence, S.C.	Yes	9-A-1
		State College, Miss.	Yes	9-A-1
		Stoneville, Miss.	Yes	9-A-1
		Tallulah, La.	Yes	9-A-1
		Waco, Tex.	Yes	9-A-1
		Tucson, Ariz.	Yes	9-A-1
ENT c1-2 (R)(C)	Development of more effective insecticides and formulations and more efficient application methods for control of the boll weevil	State College, Miss.	Yes	9-B-1
		Stoneville, Miss.	Yes	9-B-1
		Tallulah, La.	Yes	9-B-1
		Waco, Tex.	Yes	9-B-1
		College Station, Tex.	Yes	9-B-1
ENT c1-3 (R)	Physiological and nutritional research on the boll weevil	State College, Miss.	Yes	9-A-1
		Baton Rouge, La.	Yes	9-A-1
		College Station, Tex.	Yes	9-A-1
		Florence, S.C.	Yes	9-A-1
ENT c1-4 (C)	Discover and develop methods other than insecticidal for controlling the boll weevil	State College, Miss.	Yes	9-C-1 9-D-1 9-E-1 9-F-1
		Florence, S.C.	Yes	9-D-1
ENT c1-5 (C)	Discover and develop methods for eradicating the boll weevil	State College, Miss.	Yes	9-D-1
		Birmingham, Ala.	Yes	9-D-1
ENT c1-6 1/ (C)	Determine the numbers of weevils surviving the winter and period of their emergence from hibernation quarters in a 4-county area of Central Texas	College Station, Tex.	No	
ENT c2	Bollworm investigations			
ENT c2-1 (R)	Biological, physiological and nutritional research on the bollworm and tobacco budworm	Stoneville, Miss.	Yes	9-A-2
		Brownsville, Tex.	Yes	9-A-2
		College Station, Tex.	Yes	9-A-2
		Waco, Tex.	Yes	9-A-2
ENT c2-2 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the bollworm and tobacco budworm	Brownsville, Tex.	Yes	9-B-2
		Waco, Tex.	Yes	9-B-2
		College Station, Tex.	Yes	9-B-2
		Stoneville, Miss.	Yes	9-B-2
		Florence, S.C.	Yes	9-B-2
		Tucson, Ariz.	Yes	9-B-2

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ENT c2-3	Discover and develop methods other than insecticidal for controlling the bollworm and tobacco budworm	Brownsville, Tex.	Yes	9-C-2 9-F-2
		Waco, Tex.	Yes	9-C-2 9-E-2 9-F-2
		Tallulah, La.	Yes	9-C-2
		Stoneville, Miss.	Yes	9-C-2 9-F-2
		Tucson, Ariz.	Yes	9-C-2 9-F-2
		Florence, S.C.	Yes	9-E-2
ENT c2-4 (C)	Bionomics of boll weevil and bollworm populations as related to cotton insect control practices	Stoneville, Miss.	Yes	9-C-2
ENT c2-5 1/ (Gr)	The biology and ecology of spiders occurring in cotton fields of the San Joaquin Valley of California and their effects on populations of bollworms, lygus bugs, and other cotton pests	Davis, Calif.	No	
ENT c3	Cotton insects other than boll weevil, bollworm, and pink bollworm and insects attacking other fiber plants			
ENT c3-1 (R)	Biological, physiological, and nutritional research on miscellaneous insect and spider mite pests of cotton	Stoneville, Miss.	Yes	9-A-4
		Tucson, Ariz.	Yes	9-A-4
ENT c3-2	Development of more effective insecticides and formulations and more efficient application methods for control of miscellaneous insect and spider mite pests of cotton	Waco, Tex.	Yes	9-B-3
		College Station, Tex.	Yes	9-B-3
		Tucson, Ariz.	Yes	9-B-3
ENT c3-3	Discover and develop methods other than insecticidal for controlling miscellaneous insect and spider mite pests of cotton	Tucson, Ariz.	Yes	9-C-4 9-F-3
		State College, Miss.	Yes	9-F-3
		Brownsville, Tex.	Yes	9-F-3
ENT c4	Pink bollworm investigations			
ENT c4-1 (R)	Development of more effective insecticides and formulations and more efficient application methods for control of the pink bollworm	Brownsville, Tex.	Yes	9-B-4
ENT c4-8	Biological, physiological and nutritional research on the pink bollworm	Brownsville, Tex.	Yes	9-A-3
		Waco, Tex.	Yes	9-A-3
ENT c4-9	Discover and develop methods other than insecticidal for controlling or eradicating the pink bollworm	Brownsville, Tex.	Yes	9-C-3 9-D-2
ENT c5	Corn insects			
ENT c5-1 (R)	Biology and ecology of the European corn borer	Ankeny, Iowa	Yes	7-A-1 7-E-1
ENT c5-2 (R)	Chemical control of the European corn borer	Ankeny, Iowa	Yes	7-B-1 7-C-1
ENT c5-3 (R)	Plant resistance to the European corn borer	Ankeny, Iowa	Yes	7-G-1
		Wooster, Ohio	Yes	7-G-1
ENT c5-4 (R)	Biological control of the European corn borer	Ankeny, Iowa	Yes	7-D-1
ENT c5-5 (R)	Biology, ecology, and methods of control of the corn earworm	State College, Miss.	Yes	7-A-1 7-B-1
		Lafayette, Ind.	Yes	1-G-2 7-C-1 7-D-1
		Tifton, Ga.	Yes	1-B-1 1-C-5 1-D-1 1-E-6 1-F-2 1-G-2 7-A-1 7-B-1

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ENT c5-5 (R)	Biology, ecology, and methods of control of the corn earworm (cont.)	Tifton, Ga.	Yes	7-C-1 7-D-1 7-E-1 7-F-1 7-G-1
ENT c5-6 (R)	Biology, ecology, and methods of controlling miscellaneous insects attacking corn	State College, Miss.	Yes	7-A-1 7-B-1 7-C-1 7-E-1 7-G-1
		Tifton, Ga.	Yes	7-B-1 7-C-1 7-E-1
		Brookings, S.Dak.	Yes	7-E-1
ENT c5-7 (R)	Plant resistance of corn to rice weevil attack	State College, Miss.	Yes	7-G-1
ENT c5-8 (R)(C)	Biology, ecology, and methods of control of soil insects attacking corn	Brookings, S.Dak.	Yes	7-A-1 7-B-1 7-D-1 7-G-1
ENT c5-9 (C)	Distribution, biology, ecology, and control of insect vectors of corn diseases	State College, Miss.	Yes	1-H-2 7-H-1
		Tifton, Ga.	Yes	1-A-12 1-H-2 7-H-1
		Wooster, Ohio	Yes	1-H-2
AE-ENT-1 1/ (C)	Investigation of insect attraction and communication possibilities in the infrared special region	Dearborn, Mich.	No	
CR-ENT-2 1/ (Gr)	Biochemical basis for resistance of maize to attack by the European corn borer	Ames, Iowa	No	
ENT c6	Small grain insects			
ENT c6-1 (R)	Biology, ecology, and methods of control of aphids attacking small grains	Stillwater, Okla.	Yes	7-A-2
		Brookings, S.Dak.	Yes	7-B-2 7-F-2
		Tifton, Ga.	Yes	7-G-2
ENT c6-3 (R)	Biology, ecology, and methods of control of Hessian fly and wheat jointworm attacking small grains	Manhattan, Kans.	Yes	7-G-2
		West Lafayette, Ind.	Yes	7-A-2 7-G-2
ENT c6-4 (R)	Biology, ecology, and methods of control of the wheat stem sawfly	Bozeman, Mont.	Yes	7-C-2
		Minot, N.Dak.	Yes	7-B-2 7-G-2
ENT c6-5 (R)	Biology, ecology, and methods of control of insects attacking sorghums	Stillwater, Okla.	Yes	7-B-2
		Tifton, Ga.	Yes	7-B-1 7-G-2
ENT c6-6 (R)	Biology, ecology, and methods of control of soil insects and related pests of small grains	Brookings, S.Dak.	Yes	7-A-2 7-E-2
ENT c6-7 (R)	Distribution, biology, ecology, and control of insect and mite vectors of small grain diseases	Brookings, S.Dak.	Yes	7-H-2
		Manhattan, Kans.	Yes	7-H-2
		Baton Rouge, La.	Yes	8-B 8-C 8-D
ENT c6-8	Biology, ecology, and methods of control of rice field insects	Baton Rouge, La.	Yes	8-A 8-B
ENT c6-9 (C)	Biology, ecology, and methods of control of Oulema melanopa attacking small grains	Lafayette, Ind.	Yes	7-D-2 7-G-2
		East Lansing, Mich.	Yes	7-A-2 7-B-2 7-E-2 7-G-2
ENT c6-10 1/ (Gr)	Behavior of cereal leaf beetle as affected by climatic factors	Lafayette, Ind.	No	
ENT c6-11 1/ (Gr)	Microbiology and pathologies of Oulema melanopa (L.)	Columbus, Ohio	No	

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ENT c6-12 1/ (Gr)	Control of damage by larvae of the rice water weevil (Lissorhoptrus oryzophilus Kuschel) by increasing plant tolerance	Fayetteville, Ark.	No	
		Stuttgart, Ark.	No	
ENT c7	Sugarcane insects			
ENT c7-1 (R)	Biology, ecology, and methods of control of borers attacking sugarcane	Houma, La.	Yes	11-A-2 11-B-2 11-D-1 11-F-2
		Canal Point, Fla.	Yes	11-A-2 11-D-1
		Mayaguez, Puerto Rico	Yes	11-A-2 11-D-1 11-E-1
ENT c7-2 (R)	Biology, ecology, and control of insects other than borers attacking sugarcane	Houma, La.	Yes	11-A-2 11-B-2
ENT c7-3 (R)	Biology, ecology, and methods of control of insect and mite vectors of sugarcane diseases	Houma, La.	Yes	11-H-1
ENT c7-4 1/ (Gr)	Factors affecting the efficiency of Trichogramma spp. as parasites of lepidopterous pests	Baton Rouge, La.	No	
ENT c8	Legume and grass insects			
ENT c8-2 (R)	Biology, ecology, and methods of control of insects attacking legumes other than alfalfa and clovers	Columbia, Mo.	Yes	6-A-1 6-D-1 6-E-1
		Tifton, Ga.	Yes	6-A-1 6-B-1 6-D-1,2
ENT c8-3 (R)	Biology, ecology, and methods of control of insects attacking grasses	Mesa, Ariz.	Yes	5-A-4
		Lincoln, Nebr.	Yes	5-A-4
		Tifton, Ga.	Yes	5-A-4 5-B-3 5-E-1 5-G-10
		Forest Grove, Oreg.	Yes	5-A-4
		University Park, Pa.	Yes	5-A-4 5-G-6
ENT c8-4 (R)	Insect vectors of pathogenic agents affecting legumes and grasses	Columbia, Mo.	Yes	6-E-1
ENT c8-5 (R)	Insecticide residues on forage crops	Tifton, Ga.	Yes	5-C-1,2, 3,9 6-C-1,2
		Beltsville, Md.	Yes	5-C-4,5,6
		Yakima, Wash.	Yes	5-C-7,8
ENT c8-6 (C)	Biology, ecology, and methods of control of aphids, leafhoppers, seed chalcids, and miscellaneous insects attacking alfalfa	Mesa, Ariz.	Yes	5-A-2 5-B-2 5-D-4 5-G-7,8
		Tucson, Ariz.	Yes	5-G-3,4,5
		Lincoln, Nebr.	Yes	5-A-2 5-B-2 5-G-1,4
		Manhattan, Kans.	Yes	5-G-4
		University Park, Pa.	Yes	5-G-1,6
ENT c8-7	Biology, ecology, and methods of control of insects attacking clover and sweetclover	Lincoln, Nebr.	Yes	5-A-3 5-G-9
ENT c8-8	Biology, ecology, and methods of control of the alfalfa weevil	Mesa, Ariz.	Yes	5-D-3 5-G-3
		Tucson, Ariz.	Yes	5-A-2 5-G-3
		Beltsville, Md.	Yes	5-A-2 5-B-2 5-D-2

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ENT c8-8	Biology, ecology, and methods of control of the alfalfa weevil (cont.)	Beltsville, Md.	Yes	5-E-2 5-F-2 5-G-2
		Lincoln, Nebr.	Yes	5-D-2
ENT c8-9 1/ (C)	Mass production and distribution of Neodusmetia sangwani, a parasite of the Rhodesgrass scale	Weslaco, Tex.	Yes	5-D-6
ENT c8-10 1/ (Gr)	Ovipositional behavior of the alfalfa seed chalcid to chemicals occurring naturally in alfalfa	Laramie, Wyo.	Yes	5-E-3
ENT c8-11 1/ (Gr)	Attractants and stimulants for the alfalfa weevil	Blacksburg, Va.	Yes	5-E-2
ENT c8-12 1/ (Gr)	Resistance of alfalfa plants and varieties to tarnished plant bug and other mirid species (lygus bugs)	Manhattan, Kans.	Yes	5-G-7
CR-ENT-1 1/ (Gr)	Nature of resistance of Melilotus infesta to sweetclover weevil	Lincoln, Nebr.	Yes	5-G-9
ENT c9	General feeder insects			
ENT c9-1 (R)	Biology, ecology, and biological methods of control of armyworms and cutworms	Baton Rouge,La.	Yes	5-D-5
ENC c9-2 (R)	Biology, ecology, and methods of control of grasshoppers	Mesa, Ariz.	Yes	5-A-1 5-D-1
		Bozeman, Mont.	Yes	5-A-1 5-B-1 5-D-1 5-F-1
		Columbia, Mo.	Yes	5-D-1
ENT c9-3 (R)	Biology, ecology, and methods of control of white-fringed beetles	Gulfport, Miss.	Yes	5-A-5 5-B-4
ENT c9-4 1/ (Gr)	The development of artificial rearing techniques for the white-fringed beetle	Auburn, Ala.	Yes	5-A-5
ENT c10 (R)	Etiology of bee diseases and development of control methods for diseases and pests	Baton Rouge, La.	Yes	18-C-1,2
		Beltsville, Md.	Yes	18-C-2,3,4
		Laramie, Wyo.	Yes	18-C-2,5,6,7,8
		Tucson, Ariz.	Yes	18-C-4
ENT c10-2 (R)	Biology and breeding for improvement of the honey bee	Baton Rouge, La.	Yes	18-A-1,2,3,4,5,6
ENT c10-3 (R)	Behavior and utilization of honey bees in the pollination of agricultural and other economic crops	Tucson, Ariz.	Yes	18-D-1,2,4
		Baton Rouge, La.	Yes	18-D-3
ENT c10-4 (R)	Biology and utilization of insects other than honey bees in the pollination of agricultural crops	Logan, Utah	Yes	18-E-1
ENT c10-5 (R)	Effect of pesticides, insect diseases, and farm practices on honey bees and other pollinating insects	Baton Rouge, La.	Yes	18-F-1
		Laramie, Wyo.	Yes	18-F-2
		Madison, Wis.	Yes	18-F-3
ENT c10-6 1/	Management for improvement in productivity of honey bees	Madison, Wis.	Yes	18-B-1,2,3,4,5,6,7,9
		Logan, Utah	Yes	18-B-8
ENT c10-7 1/ (Gr)	Pathogenesis and diagnosis of Nosema disease in Apis mellifera L.	Columbus, Ohio	No	
ENT h1	Mosquitoes, sand flies, and gnats investigations			
ENT h1-1 (R2)	Development of more effective insecticides and other materials and methods for controlling mosquitoes	Gainesville, Fla.	Yes	13-B-1 13-D-1 13-E-1 14-B-1 14-D-1 14-E-1 16-B-1 16-D-1 16-E-1 17-B-1

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ENT h1-1 (R2)	Development of more effective insecticides and other materials and methods for controlling mosquitoes (cont.)	Gainesville, Fla.	Yes	17-C-1
		Corvallis, Oreg.	Yes	13-A-1
				13-B-1
				13-E-1
				14-A-1
				14-B-1
				14-E-1
				16-B-1
				16-E-1
				17-B-1
				17-D-1
		Fresno, Calif.	No	
		Lake Charles, La.	Yes	13-D-1
				14-D-1
				16-D-1
				17-C-1
ENT h1-4 (R2)	Studies on the distribution, abundance, taxonomy, and biology of mosquitoes affecting agriculture	Gainesville, Fla.	Yes	13-A-1
				13-E-1
				14-A-1
				14-E-1
				16-A-1
				17-A-1
				17-C-1
				17-D-1
		Corvallis, Oreg.	Yes	13-A-1
				13-E-1
				14-A-1
				14-E-1
				16-A-1
				17-A-1
				17-D-1
		Fresno, Calif.	No	
		Lake Charles, La.	Yes	13-A-1
				14-A-1
				16-A-1
				17-A-1
				17-C-1
ENT h1-5 (R2)	Development of repellents and other materials and methods to protect man and animals from mosquitoes, sand flies, and gnats	Gainesville, Fla.	Yes	13-E-1
				14-E-1
				17-D-1
		Corvallis, Oreg.	Yes	13-B-1
				14-B-1
				16-E-1
ENT h1-15 (R2)	Studies on the relationship of water and land management procedures to mosquito breeding in water impoundments and in irrigated farming areas	Corvallis, Oreg.	No	
		Fresno, Calif.	No	
ENT h1-16 (R2)	Studies on the biology and control of black flies, sand flies, and other gnats and their relationship to disease transmission, especially on livestock and poultry	Denver, Colo.	Yes	15-E-1
		Gainesville, Fla.	Yes	17-A-3
ENT h1-17 (C)	Studies on the biology and control of salt-marsh and rice-field mosquitoes in Louisiana and other Gulf Coast areas	Lake Charles, La.	Yes	13-A-1
				14-A-1
				16-A-1
				17-A-1
		Lafayette, La.	Yes	13-A-1
				14-A-1
				16-A-1
				17-A-1
ENT h1-18 1/ (C)	The effect of predators and parasites on the breeding potential of mosquitoes found in coastal-marsh areas of Louisiana	Lake Charles, La.	No	
ENT h1-19 1/ (Gr)	Population dynamics, sterilization, and attractants for the eye gnat, Hippelates pusio Loew	Gainesville, Fla.	No	

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ENT h1-20 1/ (C)	Dispersal of concentrated or undiluted insecticides for increased effectiveness and economy in mosquito control	Berkeley, Calif.	No	
ENT h1-21 1/ (C)	Pathogens as biological control agents for mosquito larvae	Berkeley, Calif.	No	
ENT h2	Investigations on flies affecting man and livestock			
ENT h2-1 (R2)	Development of insecticides, repellents, and other materials and methods for the control of horn flies, stable flies, and face flies	Kerrville, Tex.	Yes	13-A-3,5 13-B-3,5 13-E-5 14-A-3,5 14-B-3,5 14-E-5
		Corvallis, Oreg.	Yes	13-B-5 14-B-5
		Lincoln, Nebr.	Yes	13-A-4 13-B-4 13-D-2 14-A-4 14-B-4 14-D-2
		Stoneville, Miss.	Yes	13-B-5 14-B-5
		Beltsville, Md.	Yes	13-A-4 14-A-4
		Gainesville, Fla.	Yes	13-A-3 13-B-3 14-A-3 17-A-3 17-B-3
ENT h2-5 (R2)	Development of improved larvicides and other materials and methods for the control of screw-worms and fleeceworms	Mission, Tex.	Yes	13-B-6 13-E-6 14-B-6 14-E-6 15-B-1
ENT h2-7 (R2)	Studies of irradiation and radioactive insecticides on flies and other arthropods affecting man and animals	Mission, Tex.	Yes	13-A-6 14-A-6 15-A-1 15-D-1
		Corvallis, Oreg.	Yes	13-B-1 14-B-1 16-A-2 16-B-1 16-E-2 17-B-1
		Gainesville, Fla.	No	
		Kerrville, Tex.	No	
ENT h2-9 (R2)	Development of insecticides, attractants, and other materials and methods for the control of house flies and blow flies	Gainesville, Fla.	Yes	13-A-2 13-B-2 13-E-2 14-A-2 14-B-2 14-E-2 16-A-2 16-B-2 16-E-2 17-A-2 17-B-2 17-D-2
		Corvallis, Oreg.	Yes	13-A-2 13-B-2 13-E-2 14-A-2

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ENT h2-9 (R2)	Development of insecticides, attractants, and other materials and methods for the control of house flies and blow flies (cont.)	Corvallis, Oreg.	Yes	14-B-2 14-E-2 16-A-2 16-B-2 16-E-2 17-A-2 17-B-2 17-D-2
		Kerrville, Tex.	Yes	16-B-2
		Lincoln, Nebr.	No	
ENT h2-11 (R2)	Studies on the biology and control of horse flies and deer flies as they relate to pests of animals and vectors of disease	Stoneville, Miss.	Yes	13-A-8 13-B-8 13-D-3 14-A-8 14-B-8 14-D-3
		Corvallis, Oreg.	No	
		Kerrville, Tex.	Yes	13-D-3 14-D-3
ENT h2-14 (R)	Development of repellents and other methods to protect man from horse flies, deer flies, and stable flies	Gainesville, Fla.	No	
ENT h2-15 (R)	Development of improved media and mass rearing and distribution techniques for screw-worm control	Mission, Tex.	Yes	13-A-6 14-A-6 15-A-1 15-D-1
ENT h2-16 (R)	Development of attractants and other materials and methods for estimating and controlling natural screw-worm populations	Mission, Tex.	Yes	13-A-6 13-E-6 14-A-6 14-E-6 15-D-1
ENT h2-17	Development of physical and mechanical methods of controlling flies and other pests of livestock	Beltsville, Md.	Yes	13-E-2,3,4 14-E-2,3,4 16-E-2
ENT h2-18 1/ (C)	Insecticidal methods for controlling the dog fly (Stomoxys calcitrans (Linnaeus)) in the Gulf Coast area of Northwestern Florida	Panama City, Fla.	No	
ENT h2-19 1/ (Gr)	Effect of predacious mites in reducing fly production from poultry droppings	Berkeley, Calif.	No	
ENT h2-20 1/ (Gr)	Basic biology and behavior of Tabanids (horse flies)	Laramie, Wyo.	No	
ENT-0-0-1 (AID)	Studies on the biology and control of tsetse flies in Africa	Salisbury, Rhodesia	Yes	17-D-5
ENT h3(R)	Cattle grub and bot fly investigations			
ENT h3-1 (R2)	Development of new insecticides and other materials and methods for the control of grubs and bots affecting livestock	Kerrville, Tex.	Yes	13-A-7 13-B-7 14-A-7 14-B-7 15-B-3
		Corvallis, Oreg.	Yes	13-B-7 14-B-7
ENT h4	Lice, mites, ticks, and fleas affecting man and animals investigations			
ENT h4-1 (R2)	Development of improved insecticides and other materials and methods for the control of lice affecting livestock	Stoneville, Miss.	Yes	13-B-10 14-B-10
		Kerrville, Tex.	Yes	15-B-2
		Lincoln, Nebr.	Yes	13-B-10 14-B-10
ENT h4-3 (R2)	Development of improved materials and methods for the control of external parasites of poultry	Corvallis, Oreg.	Yes	16-A-3 16-B-3
		Kerrville, Tex.	Yes	16-B-3

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ENT h4-7 (R)	Development of insecticides and other methods for the control of human lice and itch mites affecting man	Gainesville, Fla.	Yes	17-B-5
ENT h4-8 (R)	Development of insecticides and other methods for the area control of ticks, mites, and fleas with particular reference to protecting man	Gainesville, Fla.	Yes	17-B-6
ENT h4-9 (R)	Development of repellents and other methods to protect man from mites, ticks, and fleas	Gainesville, Fla.	Yes	17-B-6 17-D-4
ENT h4-10 (R)	Development of insecticides and other materials and methods for the control of ticks and sheep ked on animals	Kerrville, Tex.	Yes	13-B-9 14-B-9
ENT h4-11	Studies on the role of lice, mites, ticks, fleas, and other arthropods in the transmission of anaplasmosis and other diseases of livestock	Stoneville, Miss. Beltsville, Md.	Yes Yes	13-F-1 14-F-1 13-F-1 14-F-1
ENT h4-12	Studies on the role of ticks and other arthropods in the transmission of equine piroplasmosis and on the development of insecticides and other means of controlling or eradicating vectors of the disease	Kerrville, Tex. Beltsville, Md.	Yes Yes	13-B-8 13-F-2
ENT h4-13 1/ (Gr)	Biological studies on the mite Neoschongastia americana (Hirst) on turkeys in Georgia	Athens, Ga.	No	
ENT h7	Toxicity and residue studies on insecticides and repellents in relation to the control of insects affecting livestock			
ENT h7-1 (R2)	Investigations relating to the acute and chronic toxicity of insecticides, repellents, and other materials to livestock	Kerrville, Tex.	Yes	13-C-1 14-C-1 15-C-2 16-C-2
ENT h7-2 (R2)	Extent of storage of insecticides in animal tissues and amount secreted in milk of dairy cattle when used for insect control	Kerrville, Tex. Beltsville, Md.	Yes Yes	13-C-1 14-C-1 15-C-1 16-C-1 13-C-1
ENT h7-4 (R2)	Development of quantitative bioassay methods for analysis of insecticidal chemical residues	Kerrville, Tex.	No	
ENT h10	Household insect investigations			
ENT h10-1 (R2)	Development of measures for the control of insects in homes	Gainesville, Fla.	Yes	17-B-4,7 17-D-3
ENT j1	Identification and classification of insects			
ENT j1-1 (R2)	Identification and classification of hemipterous insects	Washington, D.C.	Yes	20-A-5 20-B 20-C-6
ENT j1-2 (R2)(C)	Identification and classification of beetles	Washington, D.C. College Station, Tex.	Yes No	20-B 20-C-5
ENT j1-3 (R2)	Identification and classification of moths and butterflies	Washington, D.C.	Yes	20-A-4 20-B
ENT j1-4 (R2)	Identification and classification of grasshoppers and allied insects	Washington, D.C.	Yes	20-A-3 20-A-8 20-B
ENT j1-5 (R2)	Identification and classification of two-winged flies	Washington, D.C.	Yes	20-A-2 20-B 20-C-4
ENT j1-6 (R2)	Identification and classification of thrips	Washington, D.C.	Yes	20-A-7 20-B 20-C-7
ENT j1-7 (R2)	Identification and classification of hymenopterous insects	Washington, D.C.	Yes	20-A-6 20-B 20-C-2 20-C-3
ENT j1-8 (R2)	Identification and classification of mites, chiggers, and ticks	Washington, D.C.	Yes	20-A-1 20-B 20-C-1

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ENT j1-11 1/ (Gr)	Basic studies on the taxonomy, morphology, and ecology of cutworm larvae	Ithaca, N. Y.	No	
ENT j1-12 1/ (Gr)	Basic studies on the nature and taxonomic significance of morphological characters of female leafhoppers	Raleigh, N. C.	No	
ENT j2	Utilization of insect enemies in the control of insect pests and weeds			
ENT j2-6	Biological control of weeds	Albany, Calif.	Yes	21-B-1
ENT j2-7 (R)	Search for and importation of foreign parasites and predators of insect pests	Paris, France	Yes	21-A-1 21-C-1,2,4 21-D-1,3,4
ENT j2-8 (R)	Search for and importation of foreign insect enemies of weeds	Rome, Italy	Yes	21-C-7, 21-D-7,8
		Buenos Aires, Argentina	Yes	21-C-8
ENT j2-9 (C)	Receipt and distribution of foreign natural enemies of insect pests and weeds	Moorestown, N.J.	Yes	21-A-1 21-C-3 21-D-1,2,5,6,
		Albany, Calif.	Yes	21-D-7,8,9
ENT j2-10 1/ (C)	The insects that feed on rangeland weeds of foreign origin in the State of Idaho	Moscow, Idaho	No	
ENT j2-11 1/ (Gr)	The biologies and host relationships of tachinid parasites of insects in the State of Washington	Pullman, Wash.	No	
ENT j2-12 1/ (C)	A world review of parasites, predators, and pathogens introduced into new habitats against injurious insects and weeds	Riverside, Calif.	No	
ENT j2-13 1/ (Gr)	Selection and development of superior strains of predators and parasites	Columbia, Mo.	No	
ENT j2-14 1/ (Gr)	Studies on the significance of the life history and ecology of Lebia analis Dej., an important predaceous ground beetle	Fayetteville, Ark.	No	
ENT j2-15 1/ (Gr)	Insects associated with aquatic weed pests of foreign origin in Louisiana	Baton Rouge, La.	No	
ENT j2-16 1/ (Gr)	The attraction and concentration of insect predators by non-toxic chemical stimuli	St. Paul, Minn.	No	
ENT m1	Chemical investigations of products of natural origin for insect control			
ENT m1-14 (R)	Investigation of plants as sources of insecticides, synergists, insect repellents or attractants, or insect antimetabolites	Beltsville, Md.	Yes	19-A-2
		State College, Miss.	Yes	19-A-2
ENT m1-15 (R)(C)	Investigation of substances naturally occurring in insects that might be used to upset their development or reproduction or otherwise affect their vital processes	Beltsville, Md.	Yes	19-A-1
ENT m1-16 1/ (Gr)	Isolation, purification, and characterization of the sex attractant for the tobacco budworm, Heliothis virescens	Ann Arbor, Mich.	No	
ENT m1-17 1/ (Gr)	Investigation of the tobacco hornworm sex attractant	Madison, Wis.	No	
ENT m2	Chemical investigations to develop synthetic organic materials for insect control			
ENT m2-1 (R2)(C)	Preparation of synthetic organic compounds for testing as insecticides and synergists	Beltsville, Md.	Yes	19-B-2
		Gainesville, Fla.	Yes	19-B-2
ENT m2-4 (R2)	Development of chemical formulations for insect control	Beltsville, Md.	Yes	19-B-3
		Gainesville, Fla.	Yes	19-B-3
ENT m2-13 (R2)	Chemical investigations of radioactively labeled insect control agents	Beltsville, Md.	Yes	19-B-1
		State College, Miss.	Yes	19-B-1
ENT m2-15	Preparation of synthetic organic compounds for testing as insect control or eradication agents through effects other than death	Beltsville, Md.	Yes	19-B-2
		Gainesville, Fla.	Yes	19-B-2

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Area & Sub-heading
ENT m2-16	Preparation of compounds for testing as insect chemosterilants	Beltsville, Md.	Yes	19-B-1
ENT m2-17	Development of basic chemical information on insect chemosterilants	Beltsville, Md.	Yes	19-B-1
		State College, Miss.	Yes	19-B-1
		Gainesville, Fla.	Yes	19-B-1
ENT m2-18 1/ (C)	Synthesis of organic compounds for use in investigations on insect attractants and chemosterilants	Kansas City, Mo.	No	
ENT m3	Analysis of pesticides, pesticide residues, and accessory materials			
ENT m3-5 (R2)	Analysis of insect control chemicals, their formulations, and accessory materials	Beltsville, Md.	Yes	19-B-2,3
ENT m3-6 (C)	Determination of residues of insect control chemicals in plant and animal products and in soils	Beltsville, Md.	Yes	1-C-1,2, 3,4,6 2-C-2 3-C 5-C-4,5,6 10-C-1
		Tifton, Ga.	Yes	1-C-5 5-C-1,2,3 6-C-1,3
		Kerrville, Tex.	Yes	13-C-1 14-C-1 15-C-1 16-C-1
		Yakima, Wash.	Yes	2-C-2,3,4 3-C 5-C-7,8 11-C-1
ENT m4	Chemical investigations on fumigants and aerosols for control of insect pests			
ENT m4-1 (R2)	Development of formulas and dispensing equipment for aerosols to control insects	Beltsville, Md.	Yes	19-D
ENT m9	Laboratory tests to determine the effectiveness of insect control materials			
ENT m9-1 (R2)	Comparison of the toxic, attractant, arrestant, and repellent action of chemical materials to test insects	Beltsville, Md.	Yes	19-E-1
		Brownsville, Tex.	Yes	19-E-1
ENT m9-3 (R2)	Comparison of insecticidal materials in gas-propelled aerosols and space sprays	Beltsville, Md.	Yes	19-E-3
ENT m9-4	Biological evaluation of materials for insect control through effects other than death	Beltsville, Md.	Yes	19-E-2
		Brownsville, Tex.	Yes	19-E-2
ENT m10	Methods for disinsectization of aircraft (Not divided into line projects)	Beltsville, Md.	Yes	19-F
ENT m11	Development of methods of analysis for pesticides and pesticide residues			
ENT m11-2 (R)	Development of methods of analysis for insect control chemicals	Beltsville, Md.	Yes	19-C
		Gainesville, Fla.	Yes	19-C
		Tifton, Ga.	Yes	19-C
		Kerrville, Tex.	Yes	19-C
		Yakima, Wash.	Yes	19-C
ENT P 1	Insect Pathology Laboratory	Beltsville, Md.	Yes	22-A-1,2, 3,4,5 22-B-1,2, 3,4,5
ENT P 2	Insect Physiology Laboratory	Beltsville, Md.	Yes	23-A-1,2, 3 23-B-1,2, 3 23-C-1 23-E-1
ENT q1	Insect metabolism and physiology			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
ENT q1-1 1/	Studies on the metabolism of insecticides and other compounds in insects	Fargo, N. Dak.	Yes	23-D-1
ENT q1-2 1/	Studies on physiological processes specific to insects	Fargo, N. Dak.	No	
ENT q1-3 1/	Physiological studies on insect growth and development	Fargo, N. Dak.	No	
ENT q2	Radiation biology and insect genetics			
ENT q2-1 1/	Basic studies on radiation sterilization of insects	Fargo, N. Dak.	Yes	24-A
ENT q2-2 1/	Effects of mutagenetic chemicals on insect reproduction and heredity	Fargo, N. Dak.	Yes	24-B
ENT q2-3 1/	Cellular effects in insects resulting from exposure to chemical mutagens or radiation	Fargo, N. Dak.	Yes	24-C
ENT q2-4 1/	Genetics of selected economically important insects	Fargo, N. Dak.	Yes	24-D
ENT q2-5 1/ (Gr)	Investigations of changes in insect behavior and tropisms resulting from sterilizing doses of radiation	Athens, Ga.	No	
ENT r1 1/	Investigations of insect diseases			
ENT r1-1 1/	Evaluation of vertebrate toxicity and pathogenicity of insect virus diseases	Chicago, Ill.	No	
ENT r1-2 1/	Development of methods for continuous production of virus-susceptible insects and virus disease organisms	College Park, Md. New Brunswick, N.J. Columbus, Ohio	No No No	
	P. L. 480 Projects			
A6-ENT-4 1/	Biological control of citrus, tobacco, and vegetable aphids	Taiwan	Yes	21-C-10
A7-ENT-1	Investigations of parasites, predators, and pathogens of sugarcane borers in India	India	Yes	11-D-1 21-D-10
A7-ENT-2	Survey of beneficial parasites and predators of agricultural and horticultural crops in the Indian Union	India	Yes	21-C-10
A7-ENT-5 2/	Investigations of parasites, predators, and pathogens of insect pests of paddy (rice)	India	Yes	21-C-5
A7-ENT-6	Nutritional studies on the silkworm Bombyx mori L. - its requirements for vitamins and amino acids and its nutrition in relation to the mineral nutrition of its host plant, mulberry (Morus indica) and studies on the host specificity of the silkworm Bombyx mori L.	India	No	
A7-ENT-7	Survey for natural enemies of witchweed, and of water hyacinth and other weeds affecting waterways in India	India	Yes	21-C-9
A7-ENT-8	Developing methods for large-scale rearing of parasites under laboratory conditions	India	Yes	21-C-10
A7-ENT-9	Investigations of parasites, predators, and pathogens of the European corn borer and Heliothis spp. in India	India	Yes	7-D-1 21-C-6 21-D-10
A7-ENT-10	Acarine disease problem of honey bees	India	Yes	18-C-8
A7-ENT-14	Studies on the free amino acids of insect haemolymph and the accumulation of citric acids in insect tissue	India	No	
A7-ENT-17	Control of the coconut rhinoceros beetle Oryctes rhinoceros L.	India	Yes	21-C-10
A7-ENT-19	Biology, ecology, and utilization of insects other than honey bees in the pollination of agricultural crops	India	No	
A7-ENT-20	Studies of microbiology and pathology of insect pests of crop plants	India	No	
A7-ENT-24	Systematic and biological studies of Indian thrips	India	Yes	20-A-7
A7-ENT-25	Research on insect pests of maize with special reference to stalk borers	India	Yes	7-A-1
A7-ENT-26	Biology of gall midges affecting mangoes with special reference to the extent of damage	India	Yes	4-A-4

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
A7-ENT-28	Taxonomic studies of several families of Mallophaga (chewing lice)	India	No	
A7-ENT-29	A study of the taxonomy of adult and larval Bruchidae	India	Yes	20-A-9
A7-ENT-31 1/	Investigations of insect pests of sorghum and millets	India	No	
A7-ENT-33 1/	Hereditary variations in the ability of Myzus persicae to transmit potato leafroll and virus "Y"	India	No	
A7-ENT-35 1/	Biology of gall midges affecting citrus plants with special reference to extent of damage	India	Yes	4-A-4
A7-ENT-37 1/	Taxonomic survey of the hymenopterous parasites belonging to the family Ichneumonidae in India	India	No	
A7-ENT-40 1/	A study of factors affecting the dissemination of Coccinella septempunctata	India	No	
A7-ENT-42 1/	Survey for natural enemies of aphids in India	India	Yes	21-C-10
A10-ENT-5	Host plant-vector and host plant-virus relationships of the rough dwarf virus of corn and methods for control of the disease	Israel	Yes	7-H-1
A10-ENT-6 1/	Acoustic responses of the desert locust (Schistocerca gregaria), Moroccan locust (Dociostaurus maroccanus) and (Acrotylus insurbricus) (Orthoptera; Acrididae)	Israel	No	
A10-ENT-12 1/	Laboratory study of tick repellents and acaricides	Israel	No	
A13-ENT-3	Investigations on the biology of dung beetles in Korea and their role in the prevention of fly breeding in dung	Korea	No	
A17-ENT-5	Studies on indigenous natural enemies of scale insects and fruit flies	Pakistan	Yes	4-D-1
A17-ENT-7	Investigations on the natural enemies of corn borers	Pakistan	Yes	21-C-6
A17-ENT-8	Studies on the natural enemies of insect pests of rice	Pakistan	Yes	21-C-5
A17-ENT-9	Studies on the insect enemies of noxious weeds in Pakistan	Pakistan	Yes	21-C-9
A17-ENT-10	Studies on Oriental leafhoppers (Typhlocybinae)	Pakistan	Yes	20-C-6
A17-ENT-13 1/	Insects, other plant-feeding organisms or plant diseases which attack Eurasian watermilfoil	Pakistan	No	
E8-ENT-1	An investigation of the population dynamics of Calligypona pellucida (F.) and of the nature of the injury to oats and spring wheat caused by this plant hopper	Finland	Yes	7-D-2
E11-ENT-1	Control of the olive fly, (Dacus oleae (Gmelin)) with radiation or chemical sterilization procedures	Greece	Yes	4-A-2
E15-ENT-1	Study of Acarine disease of honey bees	Italy	Yes	18-C-8
E21-ENT-2	Studies on the possibility of biological control of aphids and scale insects and the effect of pesticides on the natural enemies of these pests	Poland	Yes	3-D-1
E21-ENT-3	The influence of some vitamins on the physiology of the Colorado potato beetle (Leptinotarsa decemlineata Say)	Poland	No	
E21-ENT-4	The causes and the role of diapause of insect pests	Poland	No	
E21-ENT-5	Studies on the differences in susceptibility of spider mites to acaricides and on cholinesterases in spider mites as influenced by acaricides	Poland	Yes	3-B-2
E21-ENT-6	The nature of infectious processes caused by protozoa in insects	Poland	No	
E21-ENT-7	The development, maturation and production of drones and natural mating of virgin and drone honey bees	Poland	Yes	18-A-5
E21-ENT-8	Mite fauna of orchards with special reference to the relation between phytophagous and predaceous species	Poland	Yes	3-D-2
E21-ENT-9	Insect vectors of virus diseases of various forage legumes	Poland	Yes	5-G-5 5-H-1
E21-ENT-10 1/	Studies on distance of mating flights of honey bee queens and drones	Poland	No	
E21-ENT-12 1/	The role of nematodes as factor reducing populations of insect pests	Poland	No	

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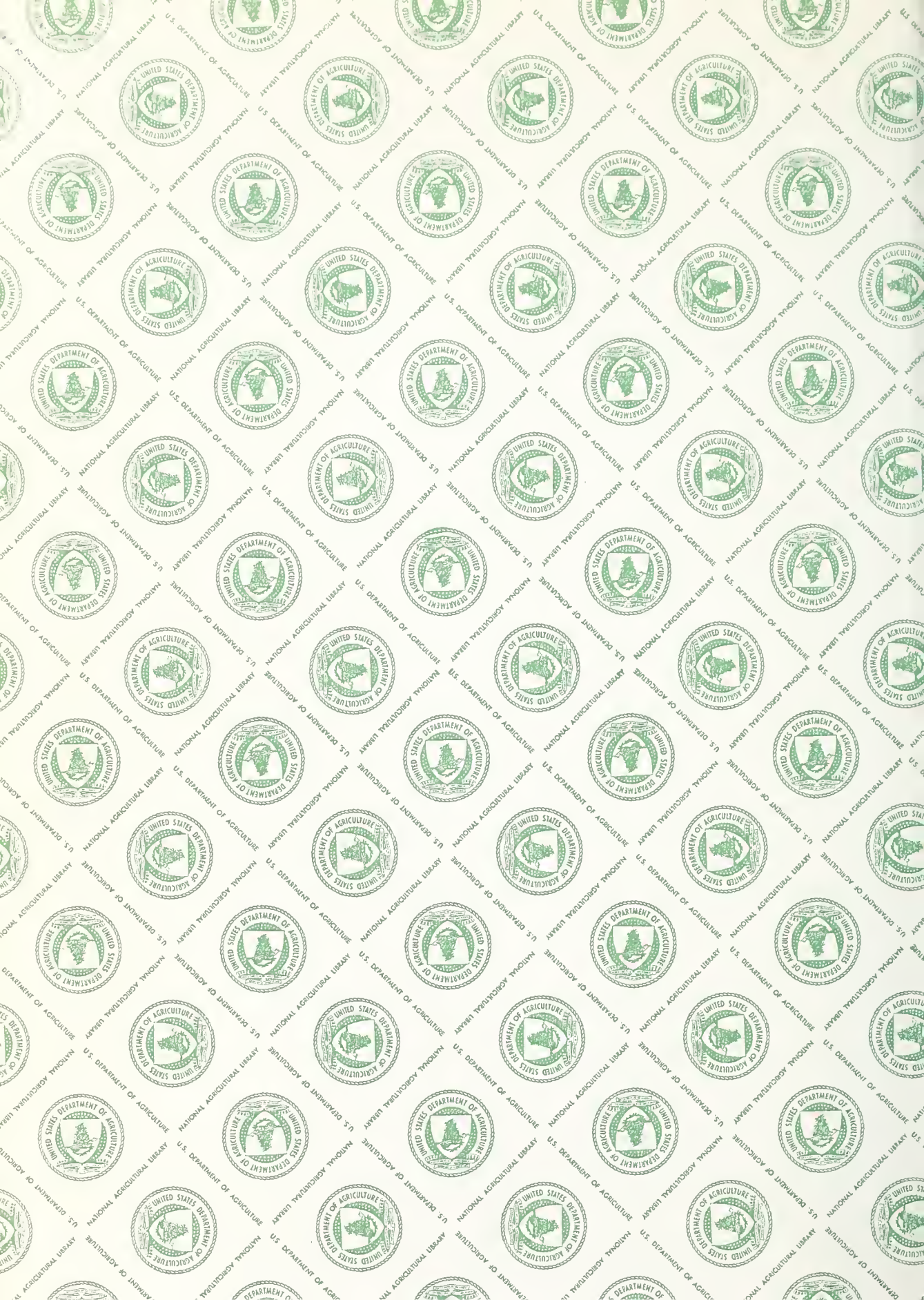
Work & Line Project Number	Work and Line Project Titles	Work Locations During Past Year	Line Proj. Incl. in Summary of Progress	Line Proj. Incl. in Area & Sub-heading
F4-ENT-2	Survey of the insect fauna of Egypt	Egypt	Yes	20-A-10
F4-ENT-3	Induced sterility in males of the Mediterranean fruit fly, Ceratitis capitata, as a means of controlling and possibly eradicating that pest	Egypt	Yes	4-A-2
F4-ENT-4	Biology, ecology and utilization of insects other than honey bees in the pollination of agricultural crops	Egypt	No	
S3-ENT-1	Biology and breeding of honey bees	Brazil	Yes	18-A-5
S3-ENT-7	Catalogue of insects living on plants in Brazil and of the parasites and predators of the insects	Brazil	Yes	21-A-3
S5-ENT-2	A biochemical study of Drosophila (vinegar flies) classification	Colombia	Yes	20-A-2
S5-ENT-3	The metabolism of temperature-acclimated Drosophila	Colombia	No	
S9-ENT-1	Studies of the parasites and predators of several insects of economic importance	Uruguay	Yes	21-A-2
S9-ENT-3	Investigations on the biology and biological control of the fire ant, Solenopsis saevissima richteri, in Uruguay	Uruguay	No	
S9-ENT-6	Systematic collections, identification, and classification of the grasshoppers of Uruguay and neighboring territories of southern Brazil, southern Paraguay, and adjacent provinces of Argentina	Uruguay	Yes	20-A-3

1/ Initiated during reporting year.
2/ Terminated during reporting year.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